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MELBOURNE

THE STRUCTURE AND FUNCTION OF THE EPIDIDYMIS

I. THE HISTOLOGY OF THE RAT EPIDIDYMIS

By B. L. Reid* and K. W. Cleland*

[*Manuscript received May* 3, 1957]

Summary

Epididymides from six white rats have been examined by standard histological techniques. The epididymis, consisting of rete, efferent ducts, and epididymal duct possesses an epithelium containing six cell types—principal, basal, ciliated, apical, halo, and clear cells—each of which is described.

Cytoplasmic differences in the principal cells permit two zones to be recognized in the efferent ducts. The principal cells along the length of the epididymal duct vary in a number of features such as: height, depth and distribution of staining, the incidence, size, and intracellular distribution of vacuoles, the shape of the nuclei, and the distribution of chromatin within them. Such differences permit definition of six major zones, some of which may be further subdivided. Variations in the histological characteristics of the various zones in different individuals have been described. These zones have been related to the usual anatomical divisions of head, isthmus, and tail.

The lumen of the epididymis contains spermatids and their breakdown products in addition to spermatozoa. The latter vary in density (number per unit volume), and in their arrangement with respect to one another, in the various zones of the efferent epididymal ducts.

The implications of these histological findings in the physiology of the epididymis are discussed.

I. Introduction

At a relatively early date the detailed anatomy of the testicular ducts had been established by the work of such men as De Graaf, Highmore, and Haller. Up to a century ago, however, nothing appears to have been known about the histology of the male ducts: the exhaustive texts of Müller (1834), Wagner (1841), Todd (1849–52), Kölliker (1853), and Hassall (1855) make no histological mention of them.

This state of affairs ended in 1856–57 with the publication of Becker's papers on the histology of the human epididymis. Three important points were established in these papers. The efferent ducts were shown to be histologically distinct from the epididymal duct not only on the basis of tubule diameter but also on the height and character of the lining epithelium. It was demonstrated that the epithelial cells of both the efferent ducts and the epididymal duct, at least in its initial part, were ciliated but that the cilia were motile only in the efferent ducts. Finally the initial part of the epididymal duct was shown to be lined by very tall cells (42–56 μ) with long cilia (35 μ) while in the distal regions of the duct both cells and cilia were much shorter.

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Apart from the work of Aigner (1900) which confirmed Becker's finding that the cilia in the epididymal duct were non-motile, and indicated that the epithelium lining the duct varied at different levels, relatively little of significance was added to our knowledge of the histology of the epididymal duct until Benoit (1926) published his monumental paper. Benoit was able to generalize Becker's third finding. In the wide range of mammals studied he showed that there was always present an initial segment in which the cells were much taller than elsewhere and that in this segment the sperm density was usually conspicuously low. He did not, however, clearly distinguish any further segments in the duct. Mietkiewski (1935, 1936), on the other hand, succeeded in differentiating six histologically distinct regions of the guinea pig epididymal duct. He was thus the first to indicate categorically and to describe clearly the very considerable histological complexity of the duct.

Some years ago one of us had occasion to examine serial sections of guinea pig testis and epididymis and was struck by the great differences in the character of the epithelium of the epididymal duct at different levels. Later, and still unaware of Mietkiewski's obscure paper, we examined guinea pig material systematically and found that the duct consists of a series of very distinct zones. Although fortuitous, it is fortunate that the guinea pig was the first species we studied, for here the zonation is much more readily appreciated than in any of the species subsequently examined. Without the case of the guinea pig constantly before us the more subtle zonation in other species could easily have been overlooked on cursory examination, as it was by earlier workers.

In the present paper it will be demonstrated by classical histological methods that the rat epididymal duct consists of six fairly distinct histological zones and that the efferent duct system is also differentiated along its length. In addition to any intrinsic morphological interest this paper will also serve as a basis of reference for certain cytological, cytochemical, and physiological studies of the rat epididymis which have yet to be published.

In the following description the term "epididymis" is used to indicate the anatomical region containing the rete (in part), the efferent duct system, and the epididymal duct.

II. Materials and Methods

Epididymides from six sexually mature individuals were examined in the present study. The fixatives used were Zenker-formol (3 specimens), Helly's fluid (1 specimen), and Aoyama's fluid with 2 per cent. mercuric chloride added (2 specimens). All samples were embedded in paraffin.

The basic facts to be described and all the quantitative data were derived from a close study of one of the above specimens (referred to as the "complete serial"). This was a testis and epididymis fixed in Zenker's fluid, double embedded by Peterfi's method using 2 per cent. collodion, and sectioned serially at 20 μ. Every section was retained. The remaining material, consisting of epididymis only, was also sectioned serially but only every 10th or 13th section (10 μ thick) was retained.

The series of complete serial sections was stained first by Newton's crystal violet method (Darlington and La Cour 1942), principally to give an adequate staining

of the sperm heads, and then with Ehrlich's haematoxylin with or without counter-staining with eosin. The remaining material, which was used to confirm the findings made on the above series and to show the normal range of variation, was stained with haematoxylin and eosin.

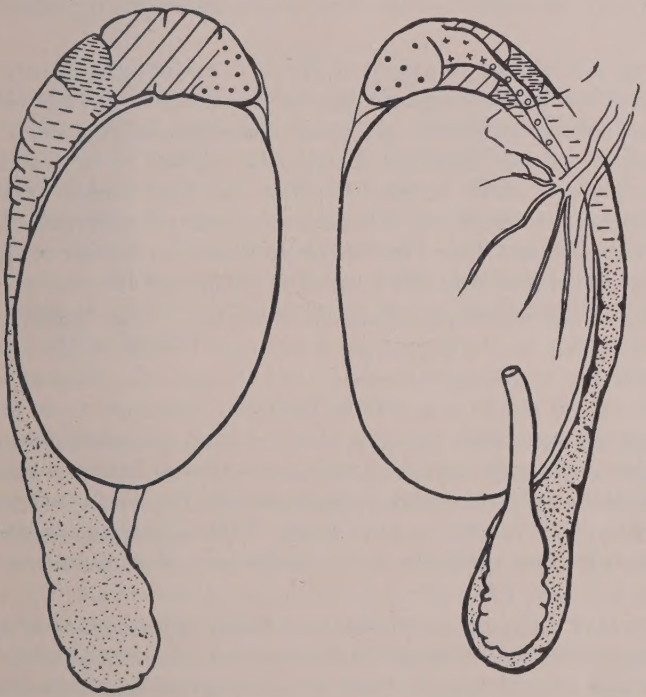

Fig. 1.—Right testis and epididymis fixed in Zenker-formol viewed from (*a*) the lateral, and (*b*) the medial aspects. In both, the cranial end is uppermost. In the medial view note the leash of testicular vessels cut close to the organ and the cut end of the deferent duct as it leaves the tail of the organ. Small circles, efferent ducts; crosses, coni vasculosi; coarse dots, zone 1; wide oblique hatching, zone 2; close horizontal hatching, zone 3; sparse horizontal hatching, zone 4; fine dots, zones 5 and 6A; unmarked, zone 6B.

III. Anatomy of the Epididymis

The testis and epididymis of the rat are so orientated in the shallow scrotum that only the ventral surface of the testis, the proximal part of the head, and the distal part of the tail of the epididymis are visible in an abdominal incision. The remainder of the epididymis lies behind the dorsal surface of the testis. Medial, lateral, dorsal, and ventral surfaces of the epididymis may thus be distinguished.

The three anatomical regions usual in mammalian epididymides are present in the rat. The recurved head is joined by the very narrow isthmus to the club-shaped tail. A thin mesentery connects all parts with the middle of the superior, dorsal, and inferior surfaces of the testis. As in other rodents, a large fat-body is associated

with, and obscures, the head of the epididymis. The details of the efferent duct connections, and some indication of the lobulation of the epididymal duct, may, however, be seen in whole preparations fixed and mounted in a suitable clearing agent (methyl benzoate for alcohol–acetic acid-fixed organs, oil of aniseed for organs fixed in Zenker-formol). Sketches from this type of preparation are reproduced in Figures 1(*a*) and 1(*b*).

Emerging from the rete at a point on the dorso-medial surface of the testis about $\frac{2}{3}$ of the distance between the equator and the superior pole, the efferent ducts pass superiorly and dorsally at first as individual, somewhat sinuous, ducts. They soon join an elongated process from the dorso-medial surface of the epididymal head proper. This process, which is separated from the head proper by a deep cleft, contains the convoluted distal ends of the individual efferent ducts and the convoluted terminal common efferent duct. The testicular vessels lie inferior to, parallel with, and only a short distance from, the leash of individual efferent ducts.

Evidence of the underlying lobulation of the head of the epididymis is visible on all surfaces. This lobulation defeats description because of its complexity, its apparent variability in different specimens, and the fact that, because of thin interlobular septa, not all the 18-or-so lobules shown by dissection to be present in the head are visible at the surface. Of note, however, is an apparently linearly arranged series of lobules most clearly seen along the ventro-superior border of the head, which contains most of the first zone of the epididymal duct. The lobules follow one another in a more or less spiral fashion, a single length of tubule joining one lobule with the next. Within any single lobule the duct appears to be sharply sinuous rather than convoluted.

Even fewer of the component lobules are visible at the surface of the tail of the epididymis than in the head. The distinction between zones 5 and 6 of the epididymal duct (defined in Section VI(*e*), (*f*)) is seen on naked-eye examination of the tail. The former zone, of moderate duct diameter, is seen to extend more inferiorly on the lateral than on the medial surface. Zone 6, of larger duct diameter, appears first near the inferior end of the lateral surface of the tail. After spreading out on the medial surface it terminates in the vas deferens at about the junction between the superior and middle thirds of this surface.

IV. Epithelial Cell Types in the Epididymis

Six distinct types of cells are found in the epithelium lining the epididymis—ciliated, basal, apical, halo, clear, and principal cells.

(*a*) *Ciliated Cells*

These cells are confined to the efferent duct system. In height they are never markedly different from the principal cells between which they lie. Their shape is usually like that of a goblet, a thin basal stem of cytoplasm connecting the expanded apical region with the basement membrane. The nuclei, which lie in the apical region and are thus above the line of basally placed principal-cell nuclei, are elongated or pear-shaped and smooth in outline. Although often rather more lightly staining than the principal-cell nuclei, they resemble the latter in the finely granular distribution

of their chromatin and in the possession of a small nucleolus. The cytoplasm of the ciliated cells lacks the granules or vacuoles which characterize the principal cells of the efferent duct system. From their smooth luminal border, immediately beneath which lies a dark row of basal granules, emerge cilia between 8 and 11 μ long.

(*b*) *Basal Cells*

Although absent in the efferent duct system, basal cells are found in the pseudostratified epithelium which lines all zones of the epididymal duct. The basal cells, which lie between the bases of the principal cells, are uniform in morphology and do not vary strikingly in density in the different zones. They are small, more or less squatly pyramidal cells, the apices of which are usually contained within the basal quarter of the epithelium. The nuclei are normally flattened against the basement membrane; they are distinguished from the nuclei of the principal cells in all but the terminal zone of the epididymal duct by the coarse chromatin granules which adhere to the nuclear membrane.

(*c*) *Apical Cells*

The pseudostratification of the first two zones of the rat epididymal duct is peculiar in that, as well as the basal cells found in all pseudostratified epithelia, there are apical cells which face the lumen but have no microscopically resolvable connection with the basement membrane. The tapering bases of these cells, which lie between the apices of the principal cells, usually terminate within the apical $\frac{2}{3}$ of the epithelium (Plate 4, Fig. 1). The cytoplasm resembles that of the principal cells in its general staining properties, its vacuole content, and its stereocilia. The nuclei of the apical cells form a layer, best seen in thick sections, in the apical half of the epithelium, well above the layer of principal cell nuclei, with which, except in shape, they are identical (Plate 1, Fig. 1). The origin and significance of the apical cells is discussed when considering the principal cells.

(*d*) *Halo Cells*

These cells, which lie between the principal cells, are found in varying numbers in the epithelium of all regions of the epididymis. They are most abundant in zones 3 and 4 of the epididymal duct where they make up as much as 25 per cent. of the total cells in the epithelium. The nuclei are smaller and more densely staining than those of the principal cells, being "amoeboid" in shape and not infrequently lobulated. The cytoplasm is very scanty and between it and the membranes of the surrounding epithelial cells lies a very lightly staining area—the "halo" (Plate 4, Figs. 3, 5, and 8).

Halo cells resemble in all essential respects the lymphocytes present in the peritubular connective tissue. They occur at all levels in the epithelium from the basement membrane to the border of the lumen but appear to be commoner in the basal regions. Halo cells have never been identified among the lumen contents and they do not appear to degenerate in the epithelium. This suggests either that they have a longer life in the epithelium than is usually assumed to be the case for tissue lymphocytes or that they are able to enter and leave the epithelium relatively freely.

(e) *Clear Cells*

Although absent in the efferent ducts and the first zone of the epididymal duct, clear cells in varying stages of activity may be identified throughout the rest of the epididymal duct. The term "clear cell" was originally applied to a conspicuously vacuolated cell present in especially great numbers (forming as much as 20 per cent. of the total epithelial cells) on either side of the junctions between zones 4A and 4B and between zones 5B and 6A. Because of their vacuolation such cells were much more lightly staining ("clear") than the neighbouring principal cells. Later it was found that these heavily vacuolated cells had a number of properties in common, and were connected by intergrades, with cells in which the vacuolation was much reduced or even absent. It is convenient to subdivide the clear cell category into three activity types based on their apparent activity as judged by the vacuole content.

(i) *Inactive Clear Cells.*—These are found especially in zones 2 and 3 and in zone 6B. As in the other types of clear cell the chromatin is finely granular, a feature which immediately distinguishes such cells from the principal cells in zone 6B (Plate 6, Fig. 1). A nucleolus, smaller than that found in the more active clear cells or in the surrounding principal cells, is present. The nuclei, which, except in zone 6B, are ovoid or elongated in the long axis of the cell and smooth in outline, are typically found higher in the epithelium than those of the principal cells, although this is less clear in zone 6B than in the earlier zones. The cell bodies are as tall as those of the principal cells except in the later parts of zone 6B but are usually smaller in volume and more deeply staining. Although stereocilia are present, the inactive clear cell lacks the distinguishing features which characterize the principal cells of the homologous zone.

(ii) *Partly Active Clear Cells.*—These occur in the early part of zone 4A, the later part of zone 4B, and the early part of zone 5B. The character and position of the nuclei are the same as in the inactive clear cells but the cytoplasm is more generous in amount, not infrequently herniating for a short distance into the lumen. The region basal to the nucleus contains vacuoles of varying sizes or hyaline eosinophilic spheres or both (Plate 4, Fig. 7; Plate 6, Fig. 6). The apical cytoplasm contains few, if any, vacuoles or spheres, being similar in staining properties, in the presence of stereocilia, but not in specific cytological differentiation, to the principal cells of the region.

(iii) *Fully Active Clear Cells.*—These are recognizable even on low-power examination (Plate 3, Figs. 1 and 3) because of the contrast between them and the much more deeply staining principal cells between which they lie. The nucleus has the same characteristics as the less active clear cells but, because of the reduced staining of the overlying cytoplasm, appears to be more lightly staining (Plate 6, Fig. 4). Both the supranuclear and infranuclear cytoplasm is occupied by large vacuoles some of which, especially in zone 6, contain lightly eosinophilic material. This may occupy either the whole vacuole or only its central regions. Stereocilia are often shorter than those of the principal cells; in the very wide cells of zone 5B and 6A they may be lacking entirely.

(f) *Principal Cells*

It is on the characteristics of the principal cells that the zonation of the epididymis, described in Section VI, is almost entirely based. Zonal differences are found in both nucleus and cytoplasm. These differences are diagrammatically illustrated in Figure 2(*d*).

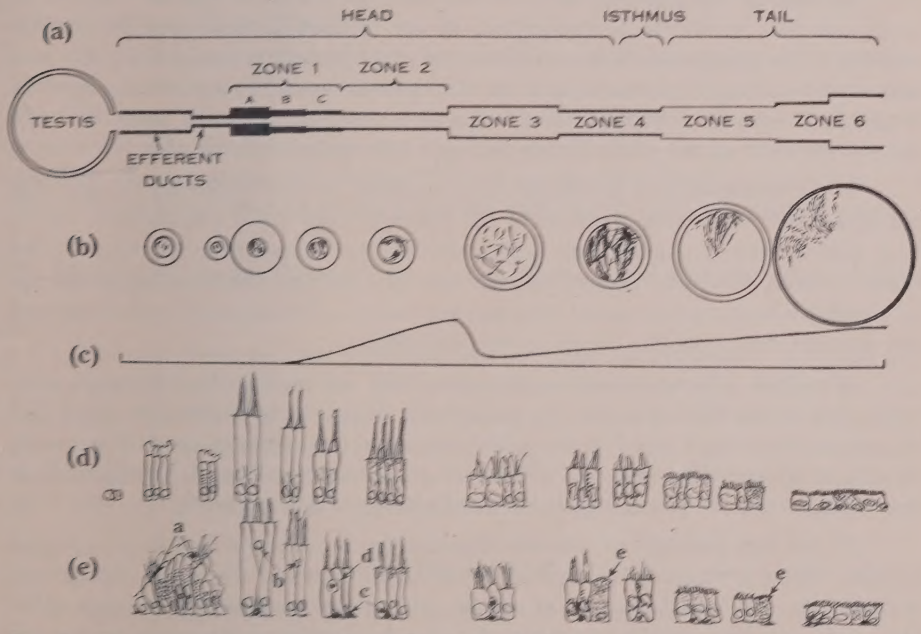

Fig. 2.—Diagrammatic representation of the principal findings. The anatomical divisions are as marked. (*a*) Scaled diagram of efferent and epididymal ducts in longitudinal section. For convenience, the lumen diameter scaling is one half that of the epithelial height. (*b*) Scaled diagram of the ducts in cross section, epithelial height and lumen diameter to the same scale. Sperm arrangement, but not relative sperm density, is also shown. (*c*) Graphical representation of the sperm density not strictly to scale. (*d*) Principal cells of the various zones. (*e*) The complete epithelium. The vacuoles of zone 4 are shown as solid bodies. *a*, Ciliated cells; *b*, apical cells; *c*, basal cells; *d*, halo cells; *e*, clear cells.

(i) *The Nuclei.*—The nuclei of the principal cells of the different zones vary considerably in overall shape (exclusive of minor irregularities of outline), outline, position in the cell, regularity of alignment with one another, arrangement of chromatin, number per cell, and mitotic rate. All principal cells possess one or two nucleoli, the largest occurring in zone 1A and the smallest in the efferent ducts and rete. There are no very striking volume differences in the nuclei of the various zones.

Mitotic figures have been seen in all zones of the efferent and epididymal ducts but are commonest in zone 1 of the latter duct. In zone 1A as many as 12 mitotic figures per 100 tubule cross sections (20 μ thick) have been found. The number is reduced in succeeding zones, being 4.2 per 100 tubule cross sections in zone 1B,

3.25 in zone 1C, 3.15 in zone 2, and between 1 and 2 in zones 3–6. Cells about to divide become rounded, lose their connection with the basement membrane, and thus come to lie in the middle or apical region of the epithelium. The spindle orientation is nearly always tangential to the tubule and the two daughter cells come to be separated by one or more non-dividing principal cells.

Because of the disparity between the number of mitoses and the number of moribund cells which can be recognized, we are inclined to look upon the mitotic activity of the principal cells as a continuation of the growth process rather than as a means of replacing dead principal cells. The growth would be a slow one, even in zone 1A: it may be calculated that in the absence of principal cell death it would take of the order of two years for the principal cells to double in number.

If this interpretation is correct there would be no valid objection to regarding the apical cells as division products of the principal cells which permanently, or for long periods, fail to regain their attachment to the basement membrane. If the mitoses merely served to replace lost principal cells, such an identification would not be possible because so few apical cells are seen between the basement membrane and the position they normally adopt in the epithelium.

Binucleate cells are common throughout the rat epididymis. Although relatively low in the efferent ducts, the percentage of binucleate cells is between 5 and 10 per cent. in zones 1 and 2 of the epididymal duct, increasing to about 25 per cent. in zone 3. In zone 4 the majority of the cells are binucleate (Plate 6, Fig. 7), while in succeeding zones the percentage is intermediate (e.g. Plate 6, Fig. 8).

In the vast majority of cells this binucleation is to be ascribed to an amitotic process. Three principal facts argue in favour of this opinion: there is no correlation between the observed frequency of mitosis and the percentage of binucleate cells; the combined volume of the two nuclei of a binucleate cell is on the whole not greatly different from that of the single nucleus in the uninucleate cell; and intermediate stages in the amitotic process are very frequently observed.

There are two principal causes for the very common irregularity of outline (as distinct from overall shape) of the principal cell nuclei: the invagination by spherical juxtanuclear vacuoles, which occurs in zone 4 (Plate 4, Figs. 2 and 4; Plate 6, Fig. 6); and the earlier stages of the amitotic process, which are found wherever binucleate cells occur, and the frequency of which is correlated with the incidence of binucleation.

The earliest stage of the amitotic process is referred to as "notching" of the nucleus. In this the nuclear membrane is invaginated into the substance of the nucleus to give a narrow notch of variable depth the sides of which are almost parallel with one another. The intermediate stage of the amitotic process is referred to as "folding" of the nucleus. In this the notching has extended deeper into the nuclear substance and the two sides of the notch, which are quite chromophilic because of adherent chromatin, are in contact with one another. The nuclear membrane is thus folded back on itself. Extension of the fold finally divides the nucleus into two more or less equal parts which are normally in close proximity to, often in contact with, one another.

(ii) *The Cytoplasm.*—The cytoplasm of the principal cells in the various parts of the epididymis varies in amount, in the depth of staining, and in the distribution of staining within individual cells; in the incidence, the size, and the intracellular distribution of vacuoles; in the presence or absence of stereocilia, in the length of the stereocilia, and the ease with which they are preserved; and in the incidence of cytoplasmic granules. Each of these factors is dealt with when considering the various parts of the epididymis.

It should be emphasised that the term "vacuole" has been used strictly in reference to the type of preparation which has been studied. There is evidence that the "vacuoles" in at least some of the zones in the epididymal duct contain lipoid material in life. The term "granule" has been restricted to bodies of about 0.3 μ or greater. The cytoplasm of a number of zones appears more finely granular than this but we have been uncertain whether this is a valid appearance or a fixation artefact.

V. Histology of the Epithelium of the Rete and Efferent Duct System

(*a*) *Rete*

The rete of the rat, the junctional part of which lies external to the testis and is thus epididymal rather than testicular, is lined by small more or less cuboidal principal cells about 8 μ in height. The nuclei of these cells are often notched and folded and binucleation is not uncommon. There is no sign of any surface differentiation of the cells or of any granules or vacuoles in the cytoplasm. Apart from some halo cells only principal cells are present in the epithelium.

(*b*) *Efferent Ducts*

The junction between the efferent ducts and the rete is a sharp one as far as the cells are concerned. On the other hand, tongues of short cells, identified as cells of the rete epithelium by the folding of their nuclei, extend for short distances into the epithelium lining the efferent ducts. On either side of these tongues a few cells of intermediate height connect the tall principal cells of the efferent ducts with the short cells of the rete. As a consequence the epithelium of this proximal junctional region of the efferent ducts has a festooned appearance.

Although there are no very striking differences in the incidence of ciliated cells along the efferent ducts, two zones may be recognized because of differences in the principal cells. The terminal end of the efferent duct system in the rat is undoubtedly a single duct, as was claimed by Benoit (1926), but we have not performed the reconstruction necessary to show an exact equivalence between the two zones about to be described and the two micro-anatomical regions of the efferent duct system, namely the individual efferent ducts and the common efferent duct.

(i) *Initial Zone.*—In the early part of this zone some degree of festooning of the epithelium is common but this cannot be shown to be due to the intrusion of rete epithelium as was the case in the short junctional zone previously described. In the more regular part of the initial zone the simple columnar epithelium averages 32 μ in height, the diameter of the duct 150 μ, and the lumen 86 μ. In the lumen, hyaline eosinophilic spheres are not uncommon.

The nuclei of the principal cells in non-festooned areas are well aligned with one another. They are usually in the basal ⅓ or ½ of the cells, are normally spherical, sometimes ovoid, and very smooth in outline because of the rarity of notching or folding. The chromatin is finely granular. In festooned areas the alignment of the often elongated nuclei is poor.

The most conspicuous feature of the cytoplasm of the principal cells is the lightly staining, finely vacuolated region which occupies from ⅓–⅔ of the supranuclear cytoplasm; where the nuclei are set higher in the cells, this region may occupy part of the infranuclear cytoplasm as well. Occasional fairly large granules are found just beneath the apical border in some cells. Around the apical border, which is angular, amoeboid, or intrudes into the lumen in the form of apocrine cupolas, we have found evidence, especially in Helly-fixed material, for the brush border described by Benoit (1926) in the efferent ducts of a number of mammals.

(ii) *Terminal Zone.*—Although the height of the epithelium in this zone (29 μ) is about the same as in the previous zone, the diameter of the tubule at the end of the zone is considerably less (95 μ). The principal cells differ from those of the initial zone in the following respects: They are more darkly staining, largely because of the absence of the supranuclear vacuolated area so conspicuous in the initial zone. In the terminal zone this region of cytoplasm contains very large numbers of small granules which are poorly stained in haematoxylin and eosin preparations but are recognizable by their refractility. The very narrow (37 μ) lumen contains few, if any, hyaline spheres.

The distal end of the terminal zone is joined directly with the proximal end of zone 1A of the epididymal duct by a short junctional segment. In this segment the ciliated cells and the granules in the principal cells are lost. The cells increase rapidly in height and soon acquire stereocilia. A much larger nucleolus appears in the nucleus, which is rather more coarsely granular than the nucleus of the efferent duct cells. Basal cells appear and rapidly become more numerous but there are, in this junctional region, neither ciliated cells nor apical cells in the apical region of the epithelium. These findings are illustrated in Figures 2*a*), 2*b*), and 2*c*).

VI. Histology of the Epithelium of the Epididymal Duct

There are marked differences, both qualitative and quantitative, in the character of the epithelium lining different regions of the epididymal duct. We are able, by purely qualitative criteria, to differentiate six major zones in the duct—4 of them in the head of the epididymis. All but two of these zones may be subdivided into two or more subzones by criteria sometimes qualitative, sometimes quantitative. The total number of such subzones is 11. They are illustrated diagrammatically in Figure 2.

In studying the zonation of the duct one is greatly helped by the fact that the epididymis is lobulated, each lobule containing a part or the whole of a subzone. In this way adjacent subzones may be directly compared within the one microscope field. The junction between successive subzones is usually fairly sharp; although the epithelium at the terminal end of one tends to be intermediate in structure, as soon as the interlobular boundary is crossed the characteristics of the succeeding subzone are very rapidly acquired.

(*a*) *Zone* 1

Zone 1, the "initial zone" of the epididymal duct, is distinguished from all other zones by the presence in the cytoplasm immediately above the nucleus of a discrete, finely vacuolated, lightly staining region which has been termed the "clear cytoplasmic area".

Within the initial zone three subzones may be distinguished. The nuclei of the principal cells of all three subzones are usually in the basal $\frac{1}{4}$ of the cell. Nearly all of them are spherical or somewhat ovoid and contain finely granular chromatin. Notching and folding of the nuclei are not conspicuous in this zone and less than 10 per cent. of the cells are binucleate. The apical border of the principal cells bulges into the lumen, especially in subzones 1A and 1B. This bulge, which lies beyond the terminal bars, is finely striated, apparently by the bases of the stereocilia. The stereocilia of zone 1 are very difficult to preserve, possibly because of their great length; this fact makes determinations of stereocilia length and (true) lumen diameter rather inaccurate.

(i) *Subzone* 1A.—This is characterized by the very tall principal cells and by the fact that the apical cells are very numerous and usually have spherical nuclei.

The diameter of the duct in this subzone is 220 μ, the epithelial height 59 μ (almost twice as high as any subzone other than 1B), the stereocilia length about 25 μ, and the lumen diameter about 52 μ. The clear cytoplasmic area occupies about the second quarter of the cytoplasm. The apical cells are so common in this subzone (ratio of principal to apical cells between 2 and 3 : 1) that they often form a fairly complete row of nuclei well above the rather ragged row of principal cell nuclei (Plate 1, Fig. 1; Plate 4, Fig. 1).

(ii) *Subzone* 1B.—This subzone is distinguished from subzone 1A by a reduction in the percentage of apical cells and by the ovoid or elongate shape of their nuclei (Plate 1, Fig. 2).

The diameter of the duct is rather less (198 μ) than in the previous zone and the epithelium is shorter (50 μ) but the diameter of the lumen and the length of the stereocilia is approximately the same.

(iii) *Subzone* 1C.—The relative shortness of the epithelium and stereocilia, the smaller number of apical cells, and especially the regular alignment of the principal cell nuclei distinguish this subzone from subzones 1A and 1B (Plate 1, Fig. 3).

The dimensions are as follows: epithelial height 33 μ, duct diameter 178 μ, length of stereocilia about 15 μ, lumen diameter about 84 μ. The nuclei, in which the nucleoli are considerably smaller than in the previous subzones, are usually ovoid and are within a few microns of the basement membrane (Plate 4, Fig. 5).

The clear cytoplasmic area is most conspicuous in the earlier part of the subzone (Plate 5, Fig. 1). Towards the end it increases in volume and is less obvious (Plate 5, Fig. 2); a few small apical cytoplasmic vacuoles may also appear. Throughout this subzone, and in succeeding zones, the apical cell border shows much less tendency to bulge into the lumen than was the case in subzones 1A and 1B.

(*b*) *Zone* 2

The distinguishing feature of this zone is the presence of vacuoles, often of considerable size, in the apical $\frac{1}{4}$–$\frac{1}{3}$ of the cytoplasm of the principal cells. These apical vacuoles are always larger than the occasional more basally placed vacuoles (Plate 5, Figs. 6 and 7). There is no clear cytoplasmic area but, as in zone 1C, the nuclei are very regularly aligned (Plate 1, Fig. 4).

The diameter of the duct (200 μ) and of the lumen (116 μ) are somewhat larger than those of subzone 1C, but the epithelial height (32–28 μ) and the length of the stereocilia (about 12 μ) are little different. Occasional apical cells with pear-shaped or elongated nuclei are present.

The nuclei of the principal cells do not differ in character, position, or regularity of arrangement from those in the previous subzone. The stereocilia in zone 2, and those of subsequent zones are often difficult to discern because they tend to be flattened against the border of the lumen by the sperm mass which occupies it; when this happens the flattened stereocilia give the impression of forming a brush border.

In the terminal lobule of this zone, the epithelium is shorter (28 μ) (Plate 2, Fig. 1). The apical vacuoles, which in the earlier regions may reach a diameter of 6 μ, although most are in the range 2–4 μ, become reduced in size and number. Indeed they may be absent from many cells. However, the presence of regularly shaped and aligned nuclei, and the absence of a well-defined clear area in the cytoplasm, distinguish this part of zone 2 from zone 3.

(*c*) *Zone* 3

The presence of the clear area which occupies most of the supranuclear cytoplasm of the short principal cells is sufficient to distinguish this zone from all others.

The diameter of the duct in zone 3 is over 50 per cent. greater (330 μ) than in previous zones. The epithelium is the lowest (19.5 μ) occurring in the head of the epididymis and the lumen diameter the greatest (about 280 μ). Stereocilia are about 6 μ long (Plate 2, Fig. 2).

The principal cell nuclei which are much less accurately aligned than those of zone 2 (Plate 6, Fig. 5) are situated just below the mid point of the cell but, because of the shortness of the cells, reach very near the basement membrane. The shape of the nuclei is irregular, varying from ovoid to more or less rectangular in optical section, the long axis in both cases usually being transverse to the long axis of the cell. The outline of the nuclei is much less regular than in previous zones; the majority are notched and many are folded.

Beneath the smooth apical border of the principal cells is a region of darkly staining cytoplasm. This lies above the clear cytoplasmic area which occupies about $\frac{2}{3}$ of the supranuclear cytoplasm (Plate 4, Fig. 3).

Towards the end of this zone some small vacuoles, more clearly defined than the very small and vague vacuoles of the clear cytoplasmic region, occur just above the nucleus in a proportion of the cells.

(*d*) *Zone* 4

This zone, occupying the distal part of the head and proximal part of the isthmus of the epididymis, is differentiated from all others by the presence in the principal cells of prominent juxtanuclear vacuoles many of which indent the nucleus. Two subzones, differing in the distribution of these vacuoles, may be distinguished.

(i) *Subzone* 4A.—The juxtanuclear vacuoles in this subzone lie in contact with the apical border of the nucleus, smaller vacuoles extending, often in rows, towards the lumen. Some trace of the clear region in the cytoplasm, so characteristic of zone 3, may be found in the early part of the subzone.

The dimensions of the duct in zone 4A are as follows: diameter 310 μ, epithelial height 26 μ, stereocilia about 5 μ, and lumen diameter about 250 μ. Although most of the principal cell nuclei have a more or less spherical overall shape, their outline is very irregular owing to notching and folding and to indentation by the juxtanuclear vacuoles. They form a ragged row somewhat below the mid point of the cells (Plate 2, Fig. 3).

The cytoplasm below and at the sides of the nucleus is haematinophilic and rarely contains vacuoles. The supranuclear cytoplasm of most cells contains large numbers of vacuoles often arranged in longitudinal rows especially near the lateral cell borders (Plate 4, Fig. 8). The largest of these vacuoles, up to 5 μ in diameter, are found in contact with the nucleus; the smallest are found towards the lumen (Plate 5, Fig. 8).

(ii) *Subzone* 4B.—In this subzone the large vacuoles in the principal cells are infranuclear or perinuclear, rather than purely supranuclear in position and the rows of vacuoles in the supranuclear cytoplasm are, depending on the position within the subzone, either less conspicuous or absent (Plate 4, Figs. 2 and 4; Plate 6, Fig. 6).

The dimensions differ little from the previous subzone (duct diameter 316 μ, height of epithelium 29.4 μ). The nuclei of the principal cells form a fairly regular row just below the mid point of the cells (Plate 2, Fig. 4). Although the nuclear outlines are rather more regular than in subzone 4A, the overall nuclear shape is less regular because of the high incidence of binucleation.

(*e*) *Zone* 5

In this zone, which begins in the distal part of the isthmus and extends down the greater part of the lateral surface of the tail of the epididymis, the principal cells lack the prominent juxtanuclear vacuoles characteristic of zone 4. The chromatin, like that of all preceding zones, occurs in finely granular form, a fact which distinguishes zone 5 from zone 6.

On the basis of differences in the cytoplasm of the principal cells, the zone may be divided into two subzones.

(i) *Subzone* 5A.—The infranuclear cytoplasm of the principal cells is here fairly generous in amount and is often more lightly staining than the supranuclear cytoplasm (Plate 3, Fig. 1).

The diameter of the epididymal duct increases from 310 μ near the beginning of this subzone to 340 μ near the end, and the epithelium is reduced in height from 24 to 20 μ.

The outlines of the nuclei are smoother than in subzone 4B because of the absence of indentation by vacuoles but their overall shape is still irregular. They form a fairly even row just below the mid point of the cells.

The infranuclear cytoplasm appears to contain very small, indistinct vacuoles (Plate 4, Fig. 7). The contrast in staining between it and the supranuclear cytoplasm, which gives the impression of being both finely vacuolated and granular, is enhanced by the pronounced eosinophilia of the latter. Part of the light staining of the infranuclear zone apparent on low-power examination of this subzone is due to the presence of more or less wedge-shaped clefts, with their apices at about the level of the nuclei, between the bases of the principal cells (Plate 4, Fig. 6).

(ii) *Subzone* 5B.—The cytoplasm of the principal cells in this subzone is homogeneously staining and the infranuclear cytoplasm very small in amount (Plate 3, Fig. 2). Basal intercellular clefts are absent. The duct diameter (340 μ) and the epithelial height (17 μ) do not vary much throughout the subzone. The length of the rather prominent stereocilia is 6 μ.

The nuclei, which form a fairly regular row somewhat below the cell mid point, are usually ovoid or rectangular in optical section, their long axis being transverse to that of the cell (Plate 6, Fig. 4). Although notching and folding are not prominent features, binucleation is still common (Plate 6, Fig. 8). Towards the end of the subzone the chromatin in the nuclei becomes more coarsely granular, being intermediate in this respect between zones 1–5 and zone 6 (Plate 6, Fig. 2).

Clear cells, which are fairly abundant throughout subzone 5B, become especially so near its distal end.

(*f*) *Zone* 6

Apart from a large and increasing lumen diameter, zone 6 is distinguished from all others by the distribution of chromatin in the principal cell nuclei. This chromatin is coarsely granular, most of the granules being attached to the nuclear membrane (Plate 6, Figs. 1, 3, and 9). The chromatin distribution is very reminiscent of that in the nuclei of the "crusty" spermatogonia and interstitial cells of the rat testis, and is almost identical with that in the nuclei of the principal cells of the early part of the vas deferens. The nuclei throughout the zone are fairly regular in shape but flattened against the basement membrane. Binucleation is not uncommon and the cytoplasm is homogeneous.

On the basis of the activity of the clear cells two subzones may be distinguished.

(i) *Subzone* 6A.—In this subzone the clear cells are abundant and very active (Plate 3, Fig. 3). The principal cells, many of which are cuboidal in shape, are shorter (14 μ) than in the previous zone and the mean diameter of the duct is much larger (430 μ). The stereocilia are short but prominent.

(ii) *Subzone* 6B.—Although clear cells are present in this zone nearly all of them are quite inactive (Plate 3, Fig. 4; Plate 6, Fig. 1). There is a gradual increase in the duct diameter to a maximum of about 620 μ. The principal cell height, which is very

variable and probably depends on the degree of contraction of the prominent muscle layer found in the wall of the duct. averages 11.5 μ: the long axis of the cells is often tangential to the duct. The stereocilia are about 3 μ in length.

VII. The Contents of the Lumen

In only two of our sets of preparations, one of them being the complete series on which the preceding description was based, are we satisfied that the loss of lumen contents during staining has been minimal. In the others only part of the lumen is occupied by sperm and what remains shows evidence of having been disturbed. In the two acceptable sets, the sperm mass completely, or almost completely, fills the true lumen, i.e. the part enclosed by the tips of the stereocilia. The following description is based on that material.

The only discrete bodies which have been identified in the lumen of the epididymis are those originating in the testis or derivatives of such bodies. No cells corresponding to the halo cells which are so common in the epithelium lining some zones of the epididymal duct have been seen. There has been no evidence—other than a short length of epithelium in an efferent duct of one specimen—for the epithelial and cellular detachment which van Eds (1934) and certain earlier workers claimed to have observed in the epididymis of a number of species including the rat. Neither has there been evidence of the masses of degenerating spermatozoa which Simeone and Young (1931) described in the vas deferens and the tail of the epididymis of the guinea pig.

The luminal bodies of testicular origin in the epididymis include both spermatozoa and immature spermatids.

(*a*) *Spermatozoa*

In different parts of the epididymis the spermatozoa vary both in density (number per unit volume) and in their arrangement with relation to one another. Figures 2(*b*) and 2(*c*) summarize these findings.

The sperm density is conspicuously low in the lumen of the rete and the initial zone of the efferent duct system. In the terminal zone of the latter the sperm density rises rapidly, then slowly increases to reach a maximum in zone 2 of the epididymal duct. After a marked decrease in zone 3 there follows a slow increase of a magnitude such that at the end of zone 6B the density is of the same order as in zone 2 (Plates 1–3).

In the efferent duct system and in zone 3 of the epididymal duct the spermatozoa are rather randomly distributed in the lumen. From the beginning of zone 1 to the end of zone 2 the spermatozoa are orientated with respect to one another: the lumen contents are arranged in areas in which either heads or tails greatly predominate (e.g. Plate 1, Fig. 4). In zones 4 and 5 and, to a lesser extent, in zone 6 the sperm are arranged in bundles, the component spermatozoa of which are all orientated in the one direction but show no tendency to the head–head and tail–tail orientation so characteristic of zones 1 and 2. These bundles sometimes adopt a more or less vortex arrangement and are separated from one another by zones containing no spermatozoa (e.g. Plate 2, Fig. 4).

(*b*) *Spermatids*

Spermatids are found in the lumen in all zones of the epididymal duct. There is, however, a marked difference in the survival of the spherical spermatids, which in the testis are unattached to Sertoli cells, and those where the nucleus is undergoing transformation into the sperm head in an extra-cytoplasmic position. The latter type of spermatid is readily distinguished from the mature sperm not only by the presence of the mass of cytoplasm but also by the presence of a thin axial filament rather than a thick sperm tail. Such spermatids occur in any numbers only in the first few zones of the epididymal duct. The spherical spermatids, on the other hand, are found in all zones.

The transforming spermatids break down in the first few zones of the epididymal duct. The nucleus is cast off and the cytoplasm, possibly after an initial swelling, fragments into a series of hyaline bodies of varying size and shape. At first lightly eosinophilic, these bodies become rather strongly haematinophilic towards the end of the duct. The identity of these two apparently different types of hyaline luminal body is indicated by their similarity in density, size, and shape and by the presence of intergrades between the two.

VIII. Variations in the Histology of the Epididymal Duct

A study of our six sets of preparations reveals that the basic zonation of the epididymal duct which has been described is present in all. Variations of a quantitative nature—lumen and duct diameters, cell height, etc.—have, of course, been encountered. While most of these are probably to be ascribed to different methods of preparation some seem undoubtedly due to genuine differences between individuals.

Perhaps the most striking quantitative variation has been in the height of the principal cells, and in the density of apical cells, in the epithelium lining zones 1A and 1B. There appears to be a positive correlation between these two characters. This is, of course, to be expected since the insertion of apical cells into an epithelium can scarcely fail to increase its height. It is unfortunate, in view of the hypothesis advanced for the origin of apical cells, that the absence of age data does not permit a test of the expected positive correlation between age and apical cell density.

Variations of a qualitative nature have been found in zone 5A. In the preparation fixed in Helly's fluid there was no significant difference between the depth of staining of the infra- and supranuclear cytoplasm. In the latter situation there was a more or less hemispherical region with its base on the lumen and its circumference almost touching the nucleus. The nucleus itself was often compressed so that its long axis lay transverse to that of the cell. It is uncertain whether this difference is due merely to the effect of different, though closely related, fixatives (Helly and Zenker–formol) or to a difference in the rate or quality of secretion in this zone in the two individual rats.

Not the least interesting of the preparations examined is one in which the lumen contains no trace of spermatozoa. In the fat-body of this azoospermic specimen are many small granulomatous lesions. The presence of a few "foam" cells suggests that these lesions may have developed in areas of earlier fat necrosis. The testis of this

animal was not grossly abnormal in size but spermatogenesis was greatly depressed and nowhere proceeded as far as mature spermatozoa. The testicular lesions are consistent with those arising from a former interference with the outflow of testicular secretion. Such interference may clearly have been the result of pressure by the lesions in the fat-body on either the efferent ducts or the proximal part of the epididymal duct.

All the zones and subzones found in normal epididymides are recognizable in the azoospermic specimen. The diameter of the epididymal duct is reduced more or less proportionately in all zones and, as would be expected, the height of the epithelium is increased (cf. Plate 5, Fig. 3, and Plate 6, Fig. 6). Although some spherical spermatids are found in the epididymal duct, especially at its distal end, the reduced diameter of the duct suggests that not even the fluid secretion, much less the cellular secretion, of the testis is anywhere near its normal rate.

The zonation of the organ and the characteristics of the various zones are, then, not contingent on a normal inflow of testicular secretion.

Some indication of the constancy of the zonal characteristics of the principal cells may be gained from Plate 5, Figures 4–7, which illustrate zone 2 in four different individuals.

IX. Discussion

Because we expect to deal with the histology of the epididymis of other species in further papers in the present series, only those studies which have been made specifically on the rat need concern us here. If, however, an assessment were to be made of the present state of studies on the epididymis, Myers-Ward's (1897) remarks would still be apposite—"While the minute structure of the testis has been the subject of numerous and extensive researches for many years, the structure and function of the epididymis and vas deferens have received, in comparison, little attention, it being largely taken for granted that these points . . . had been already settled."

The first student of the rat epididymis appears to have been Neumann (1875) who figured an unrecognizable zone of the rat epididymis in addition to rather clear figures of zone 1 in man and rabbit. Neumann noted the difference in chromatin distribution in the nuclei of basal and principal cells; he also noted the presence of binucleate cells. A supporter of von Ebner's spermatoblast theory of spermatogenesis, he made a comparison between the tall principal cells and the Sertoli cells and between the basal cells and the "Rundzellen" (modern spermatogonia, spermatocytes, and early spermatids) of the testis. He appears quite seriously to have entertained the possibility of a transformation of epididymis into active testis.

Myers-Ward (1897) included the rat among the range of species which he studied. Although misinterpreting their relation to the epithelium, he appears to have recognized apical cells. His main thesis was that "the epididymis is almost certainly a secretory tube, the secretion consisting of separated portions of the lining cells and being destined for the nourishment of the sperm cells."

The rodent epididymis is not without interest in the history of cytology because of the part it played in the development of the Henneguy-Lenhossék theory of the

relation between cilia and flagella, basal granules, and centrioles. von Lenhossék (1898) remarked that the "epididymal" epithelium (obviously the epithelium of the efferent ducts) contained "the most beautiful ciliated cells of the vertebrate body". Certainly the alternation of ciliated and non-ciliated cells in the efferent ducts permits an immediate appreciation of the reciprocal relation between basal bodies and centrioles.

In a study of a number of mammals, Henry (1900) found that the tail of the rat epididymis was lined by (stereo-) ciliated cells in the cytoplasm of which were varying numbers of different sized, intensely safranophilic granules. Where the number of granules was great the cell was found to lack (stereo-) cilia. The cells described by Henry are probably to be compared with the "clear" cells of the present paper. Henry found that the nuclei of cells about to discharge their massive secretion appeared to be poor in chromatin. As has been described in Section IV(*e*), we regard this appearance as an illusion, rather than as evidence of a relation between the nucleus and the secretion process, as Henry thought.

By far the most important paper published on the rat epididymis was that of Aigner (1900). He fully confirmed Becker's earlier finding, which subsequently had often been overlooked, that the "cilia" lining the epididymal duct were indeed non-motile, thus contrasting with the actively motile true cilia present in the ciliated cells of the efferent ducts.

In the initial part of the epididymal duct, which he clearly differentiated from the rest of the head, Aigner figured both apical and basal cell nuclei although not the cytoplasmic contours of these cells. He described and figured a highly vacuolated region of cytoplasm in the columnar cells of this part of the duct, which, although its position in the cells seems very variable, undoubtedly corresponds to our clear cytoplasmic area. He recognized that the shrunken appearance of this cytoplasmic area in his preparations was an artefact but thought it was based on some pre-existing structure.

Following the initial zone, Aigner found one in which the cells were shorter and the secretory phenomena (intracellular vacuoles, apocrine cupolas, luminal masses) were not nearly so apparent. Although Aigner overlooked the apical vacuoles, this probably corresponds to our second zone. The next zone, corresponding to our zone 3, was characterized by its wide lumen and short cells.

In the region of the isthmus Aigner found that most of the cells were filled with the finest of granules. In a quite restricted zone of the duct there were present, between the former cells, broad non-stereociliated cells in which large granules, often situated in vacuoles, were present. Clearly this zone of Aigner's corresponds to the junctional region between our subzones 4A and 4B and the broad cells with large granules are what we have called "clear cells". Only a sketchy, and to us unrecognizable, description of the tail of the epididymis is given by Aigner.

Regaud (1901) found that throughout the rat epididymis the cells contained a multitude of small granules which he was able to stain rather selectively by an old method of Weigert. We are unable to assess this finding. On the other hand, Regaud was the first to draw attention to the polymorphism and irregular outlines of the

nuclei of the rat epididymis. Like ourselves and Benoit (1926) he regarded such appearances as evidence of an amitotic process.

Apart from Benoit (1926), who dealt only sketchily with the rat and did not describe the extension of the duct beyond differentiating the first zone, the most recent paper on the rat epididymis is the primarily histochemical one of Maneely (1955).

On the basis of the distribution of periodic acid–Schiff (PAS)-reactive materials in the cells, Maneely recognized three zones in the head of the organ: an initial zone where the cytoplasm was diffusely positive, a middle region where granules above the nuclei were stained, and a lower region where massive perinuclear accumulations of positive material were present. Maneely's zones probably correspond to our zones 1–3, 4A, and 4B respectively. In his study of the tail of the rat epididymis Maneely found, especially in the mid region, certain cells which were strongly PAS-positive. These probably correspond to our clear cells.

There is some disagreement in the literature about the density of spermatozoa in different parts of the epididymis. Benoit (1926), for example, found that in many species the efferent ducts and initial zone of the epididymal duct was conspicuous for the low density of spermatozoa in the lumen, the density usually rapidly rising in the duct immediately distal to this zone. While we are able to confirm this in the guinea pig, at least in ordinary histological preparations, it is certainly not so in the rat. As has been described, the sperm density rises rapidly in the terminal zone of the efferent duct then more slowly in zones 1 and 2 of the epididymal duct. It is true that we have seen preparations which showed the type of density distribution found by Benoit, but in these there have been unmistakable signs of loss of spermatozoa during the histological processing. The wonder is that any spermatozoa survive such processing, unless double embedding has been practised those that remain must do so by virtue of their adhesion to the surface of the slide via the precipitated protein in the epididymal fluid.

This question of the sperm density in different zones of the epididymis is far from being one of purely academic interest in the histology of the organ. It is quite certain, for example, that the testis of the rat produces rather large amounts of fluid, most of which is subsequently absorbed in the epididymis. And it is an obvious inference from the histological data described in the present paper and in the papers of a series of workers beginning with van der Stricht (1893) and Myers-Ward (1897), that certain zones, at least of the epididymis, have a secretory function. Clearly the luminal sperm density could give information on both these aspects of epididymal function. On this reasoning, zone 3 of the epididymal duct is one where fluid secretion occurs. We are not, however, entirely convinced that the terminal zone of the efferent duct system in the rat is to be regarded as an absorptive zone, as the increased sperm density suggests and as many earlier workers on mouse epididymis believe (e.g. von Möllendorff 1920, Wagenseil 1928, Young 1938). This is because of the disparity between the lumen diameter (37 μ) in this zone and the length of a rat sperm (in our fixed preparations about 80 μ). Despite the presence of ciliated cells the possibility of a purely mechanical accumulation of spermatozoa—similar to the accumulation of driftwood in the narrows of a flowing stream—certainly cannot be overlooked.

It is implicit in our findings on clear cells that the epithelium of most zones of the epididymal duct is heterogeneous in its active cell population: there are, on the one hand, the principal cells which form a homogeneous group within any one zone or subzone; on the other hand, there are the clear cells which form a group, apparently homogeneous except in activity and density, extending through many zones. That these two classes of cells are indeed distinct is indicated by differences in both nucleus and cytoplasm. Thus the cytoplasm of active clear cells bears no resemblance to that of the homologous principal cells in any zone; and there are no intergrades between the two contrasting cell types. The nuclei of both active and inactive clear cells normally lie higher in the cell than the nuclei of the principal cells; and in zone 6 the chromatin distribution in both the active and inactive types of clear cell contrasts markedly with that of the principal cells.

It might be argued that the clear cells are merely degenerate principal cells or principal cells at a certain stage of their secretory cycle. Apart from the absence of intergrades between principal and clear cells, the most cogent evidence against such a viewpoint is the constancy, in both density and activity type, of the clear cells in the various zones of different individuals. Clearly, on either the degeneration or the secretory-cycle theses we would expect no such constancy. On the degeneration thesis a variation in clear cell density with age would be expected unless degeneration proceeded to death; there is no cytological evidence of such death and the mitotic rate seems too small to replace the postulated dead clear cells. On the secretory-cycle thesis one would have to assume that there was some intrazonal synchronization in the secretion cycle since the clear cells of any one zone are nearly all of the same activity type. The consequence of such a synchronization would be differences between individual rats in the density of clear cells and in their activity type; but this is not so.

Although it is evident that the clear cells are distinct from principal cells their function is not at all clear. The contents of their cytoplasmic vacuoles are sometimes stained by ordinary acid dyestuffs, a fact which would seem to exclude a mere lipid-accumulating function. Their presence in apparently normal numbers and activity types in the azoospermic specimen does not give any support to the view that their function is absorptive. If we regard the vacuoles of the clear cells as secretory in nature, the fact that these vacuoles accumulate first in the basal cytoplasm facing the blood vessels might suggest an endocrine function. Against this view is the presence of stereocilia on all but the most active of the clear cells: most of the previous workers on the epididymis have considered that the stereocilia are in some way connected with secretion towards the lumen. Obviously more work of a cytochemical and experimental nature will be needed before even a tentative function can be ascribed to the clear cells.

Detailed histological work like the present has its own intrinsic value and interest. To us not the least interest of the findings has been in the implications which they appear to have for the function of the epididymis. The known or supposed active functions of this organ seem to be: (1) the concentration of the dilute sperm suspension issuing from the testis in order both to ensure a seminal sperm concentration sufficient for fertilization and to retain the sperm in the organ for a period sufficiently long for

their final maturation (cf. Young 1929*a*, 1929*b*) to occur; (2) to provide a suitable environment for this final maturation and to maintain the sperm while it is occurring.

The impression left by the histological data is that the epididymis is over-elaborate for such relatively simple functions; this impression gains added force when it is recalled what complex functions are associated with the nephron, which is histologically rather simple when compared with the epididymis.

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Explanation of Plates 1–6

Plates 1–3

Zenker-formol-fixed complete serial sections (20 μ thick) of the epididymal duct. Plates 1 and 2 from sections stained with haematoxylin, Plate 3 stained with haematoxylin and eosin. The magnification throughout these 3 plates is 126 diameters

Plate 1

Fig. 1.—Zone 1A. Note the large number of apical cell nuclei and the raggedness of the row of principal cell nuclei. The long stereocilia reduce the true lumen to only a small proportion of the apparent lumen.

Fig. 2.—Zone 1B. The sperm density is higher than in zone 1A and the head–head and tail–tail orientation of the sperm more apparent. The apical cell nuclei are greatly reduced in number and are elongated rather than spherical.

Fig. 3.—Zone 1C. Note the regular alignment of the principal cell nuclei, the further reduction in the number of apical cells, the shorter stereocilia, and the very apparent sperm orientation.

Fig. 4.—Zone 2. There is a further reduction in the length of the stereocilia. The apical vacuoles are just visible at this magnification. An interlobular septum crosses the field of view.

Plate 2

Fig. 1.—Zone 2. This figure is from towards the terminal end of the zone. The epithelium is shorter and the apical vacuoles fewer in number and smaller in size than in Plate 1, Figure 4.

Fig. 2.—Zone 3. Note the very low epithelium. The sperm density is quite low in comparison with the preceding zones and the sperm show little or no orientation with respect to one another.

Fig. 3. Zone 4A. The epithelium is taller than in the previous zone and many halo-cell nuclei are visible in the supranuclear region. The sperm are arranged in bundles.

Fig. 4.—Zone 4B. The sperm density has increased and the number of halo cells is greatly reduced.

Plate 3

Fig. 1.—Zone 5A. Note the more lightly staining infranuclear region of the epithelium.

Fig. 2.—Zone 5B. The epithelium is shorter than in the previous subzone and the infranuclear region is not differentiated. The more lightly staining regions in the epithelium are clear cells.

Fig. 3.—Zone 6A. There is an abundance of active clear cells in the epithelium. The sperm, although still in bundles, occupy the lumen more fully than in zones 4 and 5.

Fig. 4.—Zone 6B. The epithelium is very short and the sperm density high. Note the pronounced layer of muscle around the duct.

Plates 4–6

Segments of epithelium from the different zones; fixation, section thickness, and staining variable (Z = Zenker-formol; H = Helly; AS = Aoyama sublimate, fixation; Hn = haematoxylin; HnE = haematoxylin and eosin staining). The magnification throughout is 735 diameters. Clear cells are shown at *c*

Plate 4

Fig. 1.—Zone 1A. Z, Hn, 20 μ (complete serial). Basal, principal, and apical cells visible. The clear area of the principal and apical cells is not very conspicuous because of the section thickness. Striations representing the insertions of the stereocilia are visible on the apical border of some of the principal cells. In the lumen the stereocilia are irregularly agglutinated.

Fig. 2.—Early zone 4B. H, HnE, 10 μ. The perinuclear vacuoles are conspicuous; some vacuoles still present in supranuclear cytoplasm.

Fig. 3.—Zone 3. H, HnE, 10 μ. The clear cytoplasmic area occupies most of the supranuclear cytoplasm.

Fig. 4.—As in Plate 4, Figure 2.

Fig. 5.—Zone 1C. Z, Hn, 20 μ (complete serial). Note the apical and halo cells. The clear cytoplasmic area is not very conspicuous because of the section thickness (cf. Plate 5, Fig. 1).

Figs. 6 and 7.—Zone 5A. Z, HnE, 20 μ (complete serial). The infranuclear region of the epithelium is more lightly staining partly because of the clefts between the bases of the cells, partly because of a genuine cytoplasmic staining difference. Note the partly active clear cell in Figure 7.

Fig. 8.—Zone 4A. H, HnE, 10 μ. Note the strings of vacuoles in the supranuclear cytoplasm (cf. Plate 2, Fig. 4).

Plate 5

Figs. 1 and 2.—Zone 1C (early and late respectively). Z, HnE, 10 μ. Note the expansion of the clear cytoplasmic area as the end of the zone is approached.

Fig. 3.—Zone 4B. AS, HnE, 10 μ (azoospermic specimen). The infranuclear vacuoles are prominent (cf. Plate 6, Fig. 6).

Figs. 4–7.—Zone 2, showing the constancy of the apical vacuoles in different individuals and after different fixatives. Figure 4: AS, HnE, 10 μ (azoospermic); Figure 5: Z, HnE, 10 μ; Figure 6: H, HnE, 10 μ; Figure 7: Z, HnE, 20 μ (complete serial).

Fig. 8.—Zone 4A. Z, HnE, 20 μ (complete serial). The juxtanuclear vacuoles and supranuclear strings of vacuoles are not very prominent because of the section thickness (cf. Plate 1, Fig. 4).

Plate 6

All figures are from Z, HnE, 20 μ (complete serial) preparations

Fig. 1.—Zone 6B. An inactive clear cell is seen in the middle of the epithelial strip. The muscle layer extends beyond the field of view.

Fig. 2.—Zone 5B. One fully active clear cell present.

Fig. 3.—Zone 6A. Note the contrast between the clear cell nucleus near the lower end of the strip and the principal cell nuclei.

Fig. 4.—Zone 5B. Note the clear cells.

Fig. 5.—Zone 3 (late). The clear cytoplasmic area is not very prominent at the end of this zone. Two partly active clear cells are present.

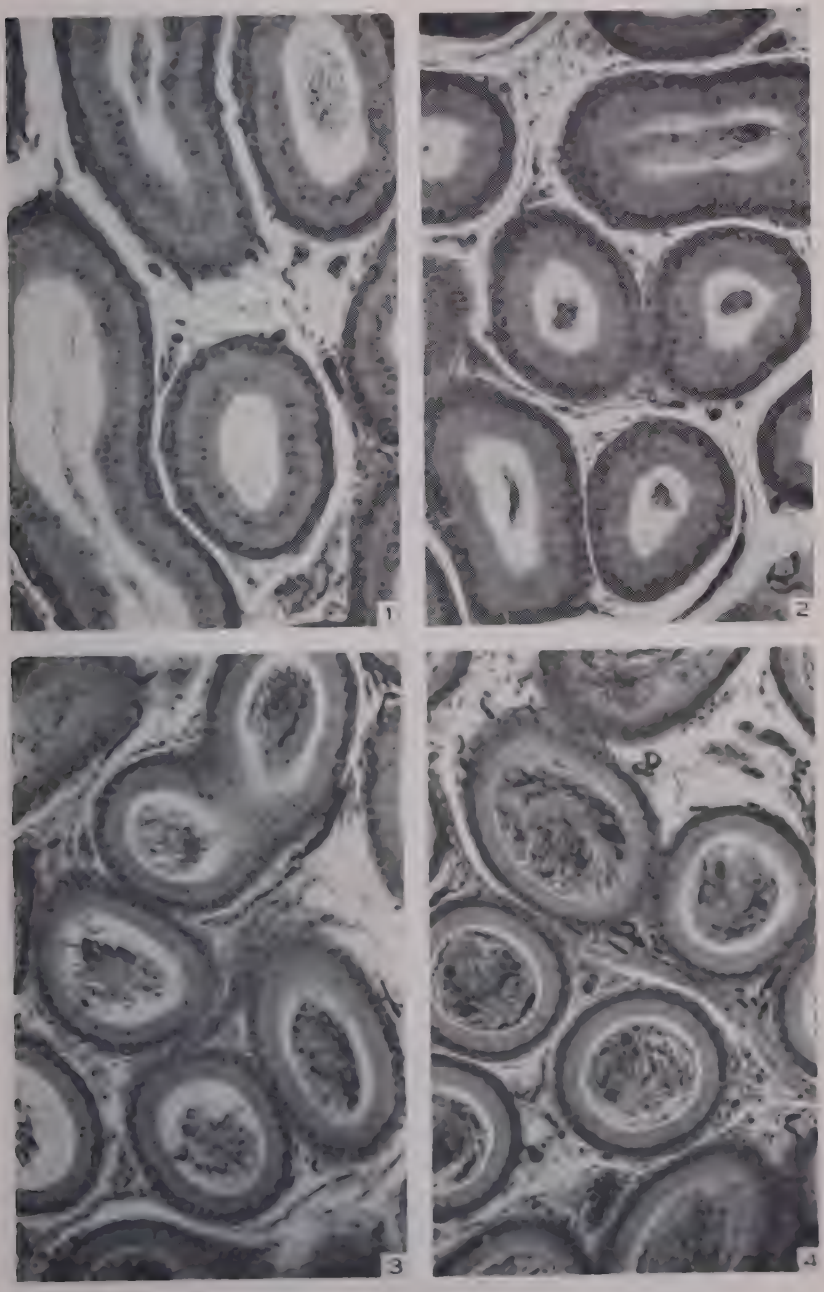
Fig. 6.—Zone 4B. Two partly active clear cells present. The juxtanuclear vacuoles are mainly infranuclear in position.

Fig. 7.—Zone 4B (tangential section). The nuclei are notched, folded, and indented by the juxtanuclear vacuoles. Most of the cells are binucleate.

Fig. 8.—Zone 5B (tangential section). The incidence of binucleation is much less than in Plate 6, Figure 7. There is no difference in chromatin distribution between the principal cell nuclei and those of the fully active clear cells (recognizable by the lightly staining cytoplasm around them).

Fig. 9.—Zone 6B (tangential section). The difference in chromatin distribution between the principal cell nuclei and the nuclei of the two inactive clear cells is marked.

THE STRUCTURE AND FUNCTION OF THE EPIDIDYMIS. I



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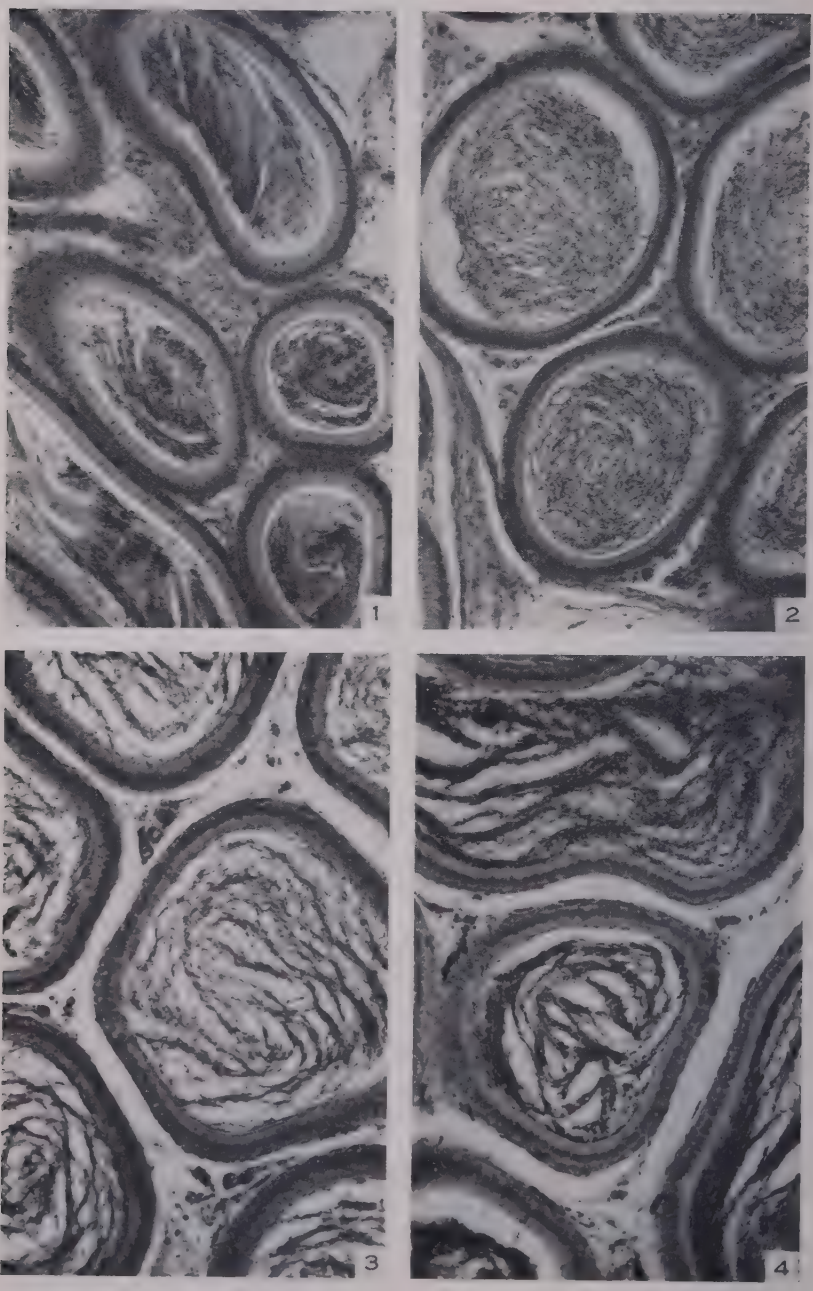

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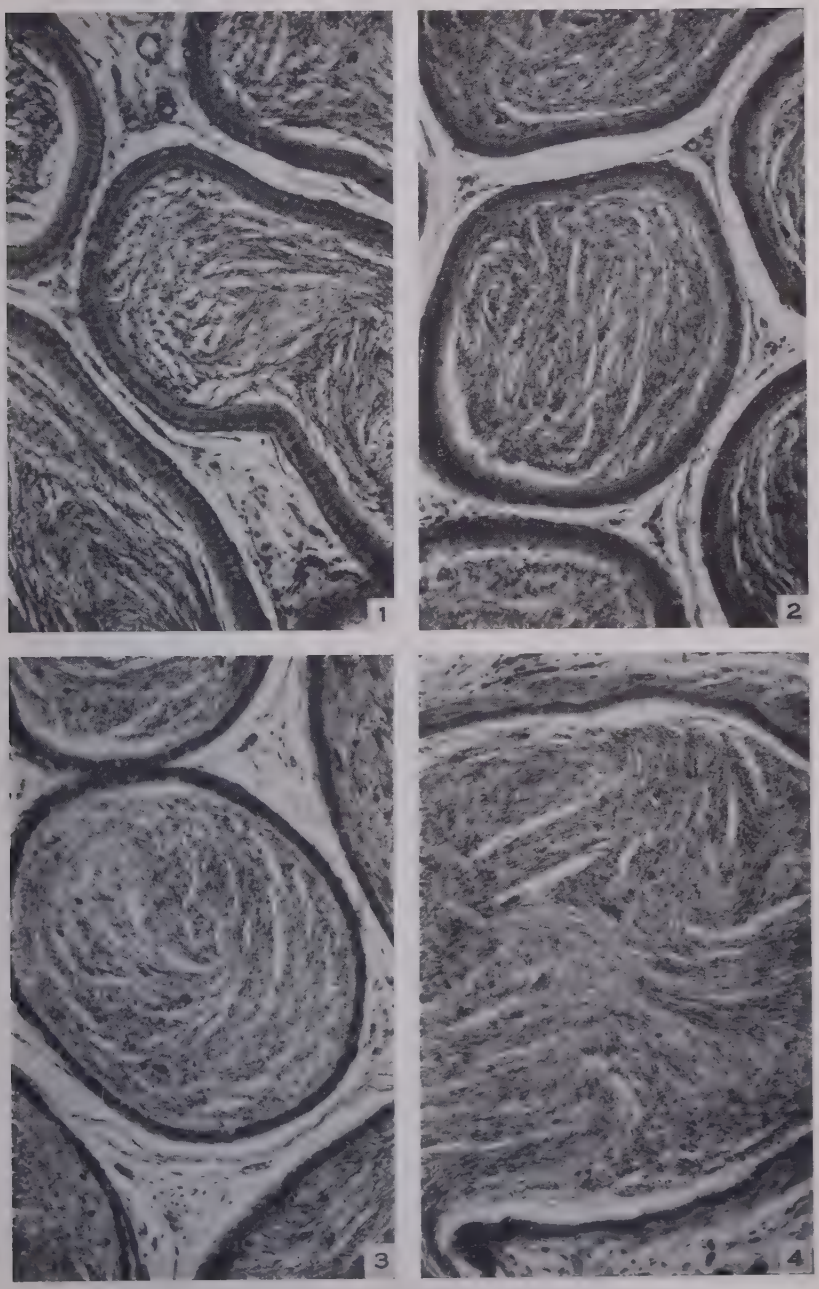

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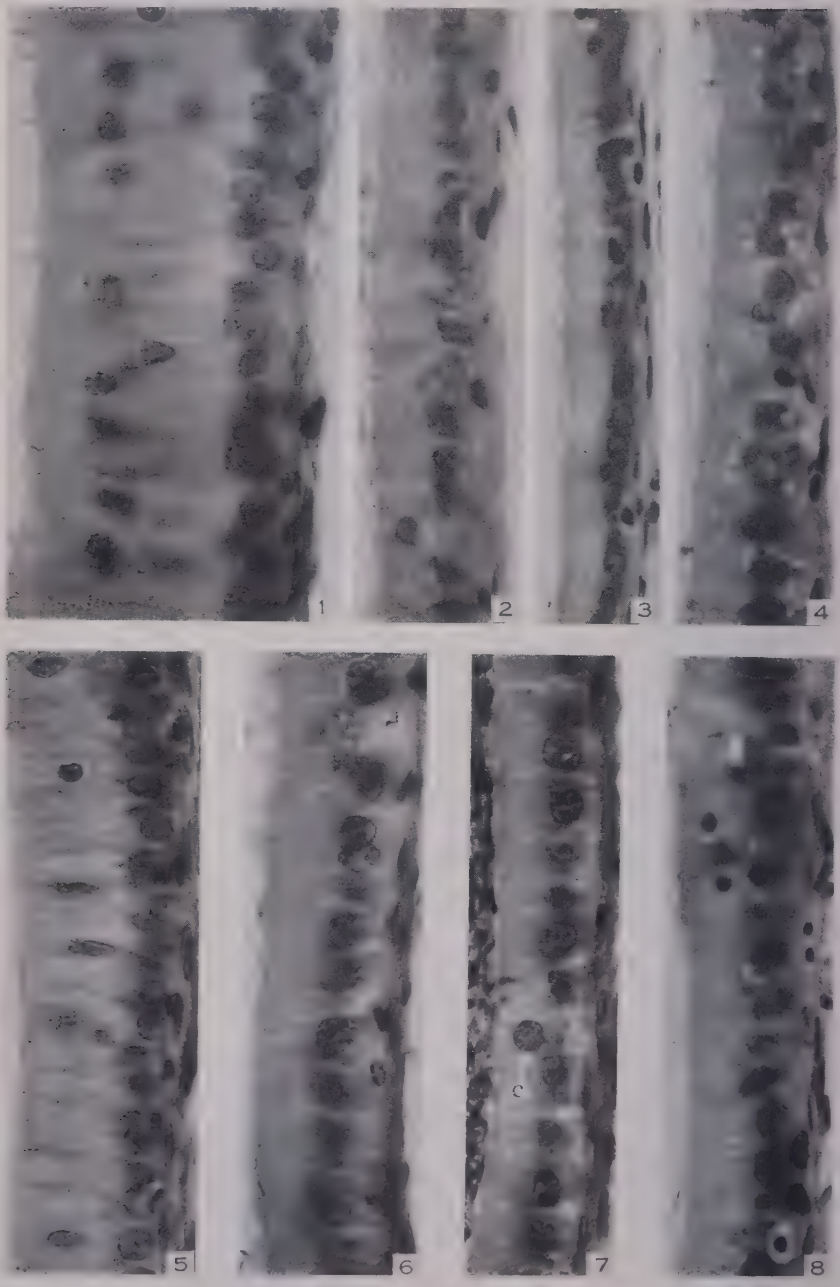

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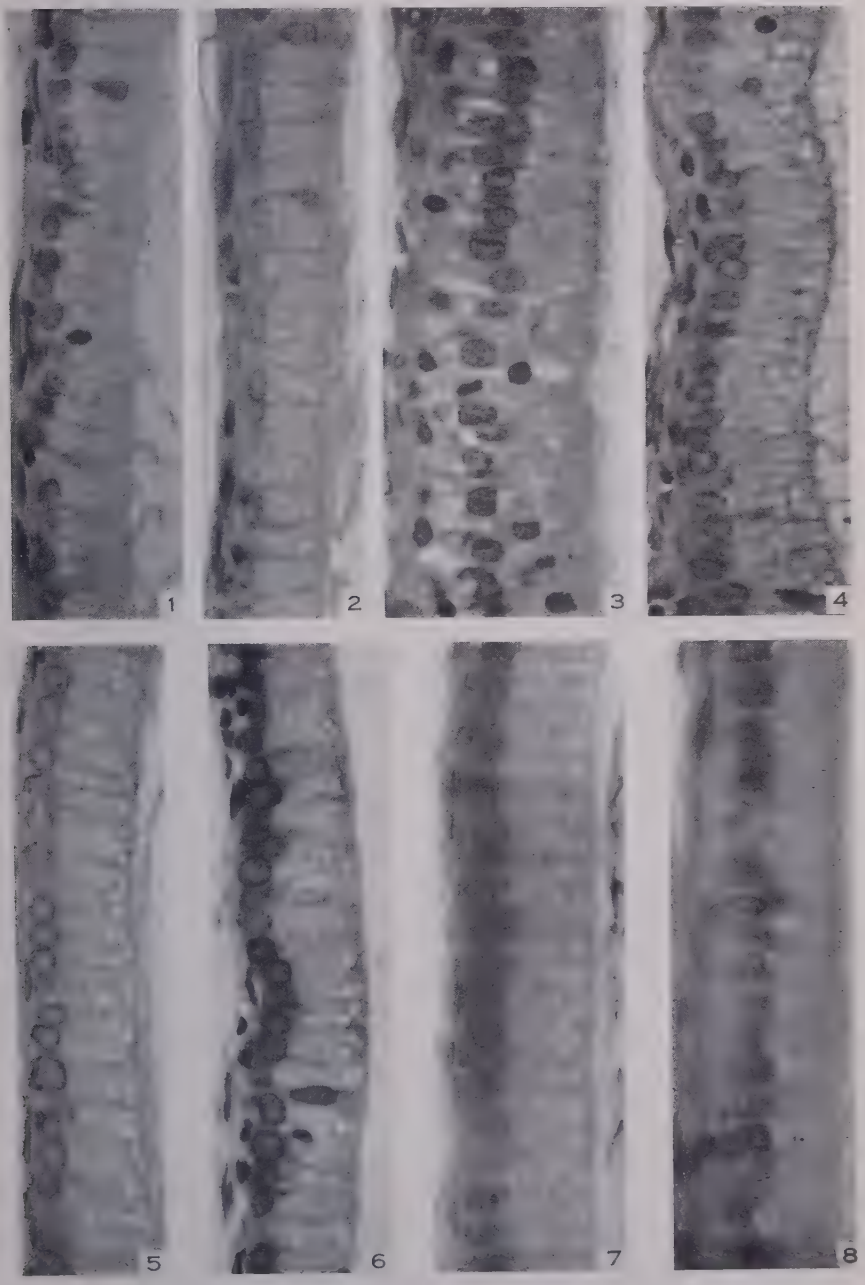

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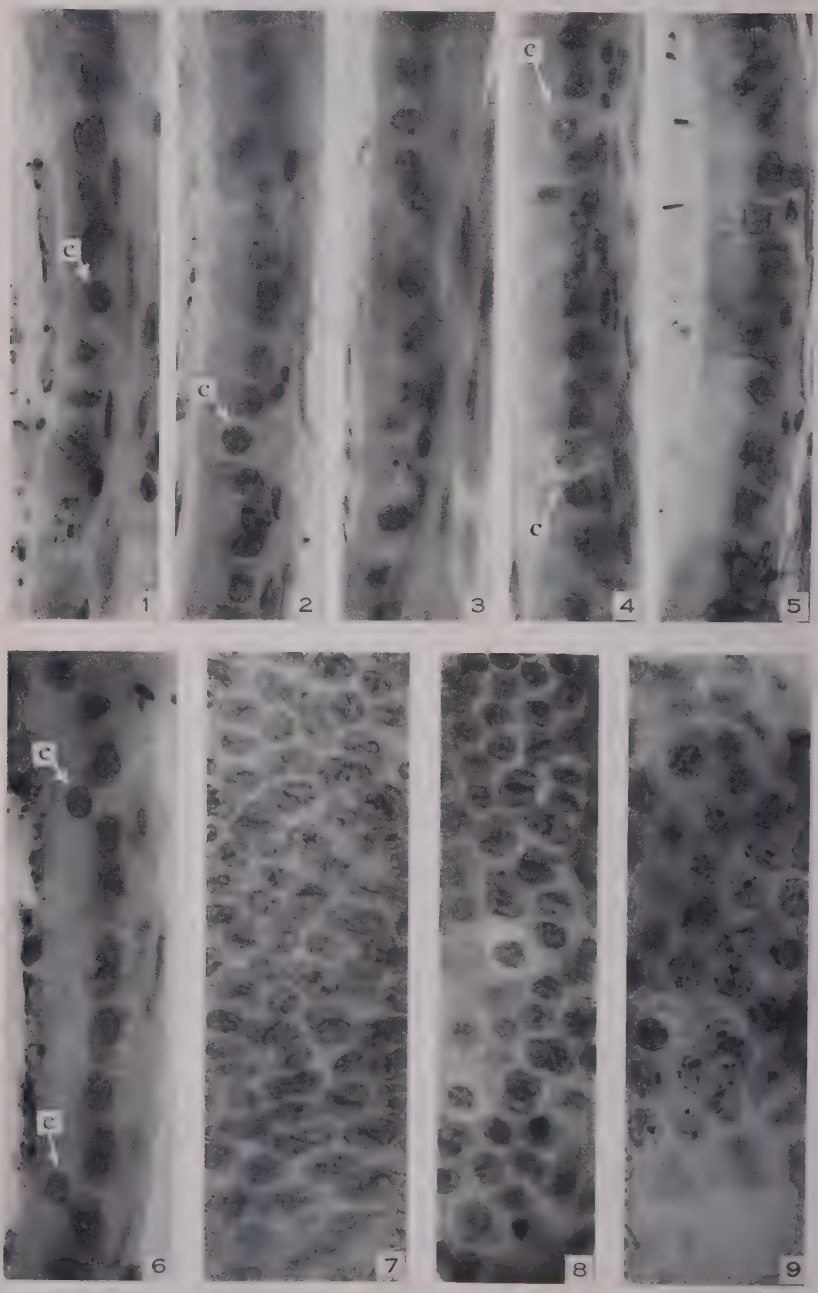

KENTROMORPHIC PHASES IN THREE SPECIES OF PHASMATODEA

By K. H. L. Key*

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Contents

Summary

Nymphs of the injurious Australian Phasmatidae *Podacanthus wilkinsoni* Macl., *Didymuria violescens* (Leach), and *Ctenomorphodes tessulata* (Gray) were reared from the early instars in crowds and in isolation. Typical kentromorphic phase differences, quite analogous to those of locusts, developed in the colour pattern of the isolated and crowded insects and persisted into the adult stage. This is believed to be the first record of kentromorphic phases in the Phasmatodea.

The two types of pattern are described and illustrated in colour. The "low-density phase" is rather uniform and usually green, while the "high-density phase" is conspicuously patterned with black, yellow, and sometimes white. The extreme conditions are connected by all intergrades and for *Podacanthus* a rating scale has been used to measure the phase level attained by individual insects.

Observations were made on the mean phase rating corresponding to different densities of *Podacanthus* nymphs on eucalypt saplings in the field. The rating rose from about 1·5 at densities of one insect per 10–20 15-in. branchlets to the scale maximum of 4·0 at densities of about one insect per branchlet. A correlation was also found between the ratings of museum specimens and subjective estimates of abundance recorded by their collectors.

All three species showed morphometric phase differences also analogous to those of locusts: the length of femur III, the ratio length of femur III/width of head, and the sexual dimorphism with respect to width of head all tended to lower values in the high-density phase.

The phasmatids showed no overt gregariousness and *Podacanthus* nymphs spent 95 per cent. of their time at rest, even in crowds of the high-density phase. Thus, in this group, activity and gregariousness can play no part in phase change. It

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is therefore probable that, both here and in locusts, the physiological mechanism of pattern change involves a direct nervous link between sensory stimulation and the endocrine system responsible for the pigmentation.

Neither Uvarov's nor Kennedy's theory regarding the biological function of phase pattern change is applicable to the Phasmatidae and it must be doubted that either is applicable to locusts. It is suggested that the colour-pattern aspect of kentromorphic variation may be a mechanism for effecting a switch-over from procryptic to aposematic adaptations in nice correlation with the population densities under which each of these has survival value.

I. Introduction

Three species of Phasmatodea, or stick-insects, may occur in plague numbers in New South Wales, where they attack, and may seriously damage, eucalypt forests. *Podacanthus wilkinsoni* Macl. has been known in this role since its original description (Macleay 1881) based on material from an outbreak near Binda Caves. Its biology has been briefly discussed by Froggatt (1905). A second species, *Didymuria violescens* (Leach), has not hitherto been recorded in the literature as a pest, although its male was figured by Froggatt (1905) as an immature stage of *P. wilkinsoni* and it must therefore have been abundant, along with the latter species, in the Nowendoc area, from which Froggatt drew his material. The third species, *Ctenomorphodes tessulata* (Gray), is also unrecorded as a pest. Its outbreaks occur in north-eastern New South Wales, at lower altitudes than the other two species. A paper by P. Hadlington, K. G. Campbell, and Mary Casimir on the biology and ecology of all three species is in preparation.

During outbreaks of *P. wilkinsoni*, the nymphs show a striking black and yellow-buff pattern reminiscent of the phase *gregaria* in the nymphs of some locusts. This pattern was noted by Froggatt (1905), who states that in the course of their development the nymphs "take on a yellow and black banded tint". Since kentromorphic* phases had never been recorded in the Phasmatodea, it was of some interest to determine whether the unusual pattern of the *Podacanthus* nymphs was an expression of this type of variation and, if so, what resemblances might exist between the phenomena in the two groups of insects. Experiments and observations here reported were undertaken with this object, and the investigation was extended to include the other two species also.

II. Taxonomy

The Australian Phasmatodea, in common with the order as a whole, are in need of a fundamental taxonomic revision. The most recent monograph by Brunner and Redtenbacher (1908) contains so many serious errors that it is of limited value to the student who is not prepared to check every conclusion against the earlier literature. Thus, in order to ensure that the names to be employed for the three species discussed here would be as soundly based as possible, the author has undertaken a taxonomic review of the genera concerned and some of their near relatives. As one result of that

* The term "kentromorphic" was introduced by Key and Day (1954) as a qualifying epithet to differentiate the "phases" of Uvarov from seasonal phases and other transient manifestations such as the temperature-induced pigmentary phases of *Kosciuscola*.

work, an application has been submitted to the International Commission on Zoological Nomenclature for the addition of the three names, along with some others, to the Official Lists of generic and specific names in zoology. Apart from purely nomenclatural issues, the existing descriptions and keys, and hence the means for identifying the species, are quite inadequate by modern standards. This situation cannot be remedied within the scope of the present paper. What is required is a comprehensive revision of the three genera concerned, with redescriptions of all the included species.

All three of the present species belong to the family Phasmatidae, *Podacanthus* and *Didymuria* to the subfamily Podacanthinae and *Ctenomorphodes* to the Phasmatinae. The three valid described species of *Podacanthus* are readily distinguished from each other. *P. wilkinsoni* may be recognized on a number of good specific characters, of which perhaps the most useful are: the orange to brownish yellow base to the pre-anal part of the wing (the coriaceous "costal field"); the shape of the operculum, which is only very gently curved for most of its length, but sharply so cephalad, and bears a few small laterocephalic teeth; and the emarginate caudal margin of the poculum.

The several species of *Ctenomorphodes* are less readily distinguished. *C. tessulata* is one of the smaller and more slender ones, its nearest known relative being the type species, *C. briareus* (Gray). The following are its more diagnostic characters: head caudad of the eyes longer than 1½ times the eye length, narrowing only very slightly caudad; tegmen of male without a whitish patch; pre-anal part of wing buff to orange-brown, without a red area proximad, anal part strongly tessellated; ventromedian carina of femora II and III unspined proximad over a distance equal to at least twice the normal spacing between spines; abdominal tergum VI not, or barely, expanded laterocaudad in the female; operculum bearing a mesocephalic knob; female cercus longer and more pointed than in *briareus*.

The position in *Didymuria* is more complicated. Material of more than 100 museum specimens available to me from many localities is very variable in a number of characters of coloration and structure, but appears to belong to a single species. In the light of this material, it seems questionable whether the three described species are distinct, especially *D. violescens* and *D. discolor* (Redt.). On the other hand, within the supposedly single species two forms may be recognized. What we may call "form 1" has a predominantly coastal distribution and the following typical constellation of characters in the male: Smaller and more slender. Antenna at least ¼ longer than femur I. Tegmen distinctly shorter than mesonotum. Wing with the pre-anal part green, cephalic margin not much paler than the rest; anal part, including the wing membrane, a strong violet. Femur III only moderately inflated, the ventromedian spines relatively weaker. Abdomen purple to red over the 4–5 more cephalic tergites; cerci subequal to, or longer than, terga IX – X; poculum more pointed and less splayed caudad. "Form 2" has a more inland distribution and the following characters in the male: Larger and more robust. Antenna less than ¼ longer than femur I, usually subequal to it. Tegmen subequal to mesonotum in length. Wing with the pre-anal part mainly buff to yellow-brown, cephalic margin very pale; anal part a paler purple-violet, most of the colour in the veins. Femur III variable, often strongly inflated,

the ventromedian spines relatively stronger. Abdomen beige to brown over the 4–5 more cephalic tergites: cerci usually slightly shorter than terga IX — X; poculum less pointed and more splayed caudad. The two forms are almost indistinguishable in the female, but form 1 tends to be somewhat more slender with a shorter tegmen, as in the male. Although both forms occur in a typical condition in the Jenolan area, N.S.W., intermediates of various types are found both there and at some other localities.

The status of these forms cannot be determined without a thorough analysis of their geographical overlap and intergradation, for which much more material is required. Rearing and breeding experiments would also be desirable. The most plausible conclusion that can be reached on the evidence at present available is the one already stated, namely that the two forms are conspecific. Now the original description and figure of *D. violescens* by Leach (1814), as well as those of Gray (1833), which were based in part on the same material, clearly refer to form 1, whereas all, or almost all, the material from economic infestations conforms to form 2. Thus, if it should prove that the two forms are specifically distinct, the name *violescens*, which is here used for both, would not be applicable to the economic species and it is not clear at the moment what name it would bear.

The suggestion naturally arises that the two "forms" of *D. violescens* are kentromorphic phases. While some element of phase variation may be involved, evidence to be presented in Section IV does not favour this explanation.

Determined specimens of both sexes of all three species have been deposited in the Division of Entomology Museum, C.S.I.R.O., Canberra, and in the British Museum (Natural History).

III. Podacanthus wilkinsoni

(*a*) *Laboratory Rearing in Isolation and in a Crowd*

During October 1954 a batch of first- and second-instar nymphs of *P. wilkinsoni*, collected some days previously at Jenolan, N.S.W., were received. These were used to set up five cultures, four comprising 1 nymph each and the fifth 19 nymphs. Cylindrical 16-mesh wire-gauze cages, 3·5 in. high by 3 in. in diameter, were employed. Each was fitted with a detachable wire-gauze floor in the centre of which was a hole of $\frac{3}{4}$ in. diam. It was stood over a jar of water, with sprigs of *Eucalyptus dives* Schauer projecting through the hole to serve as food. Each cage was screened from view from the others by sheets of cardboard; all were placed in bright diffused light at room temperature. The food was changed every few days.

Deaths and moults were recorded as they were observed in each cage and the dead bodies and skins removed. Not all moults could be recorded, since the nymphs tend to eat the skins. In consequence, difficulty arose in distinguishing the instars. Mary Casimir (personal communication) observed seven nymphal instars in this species. A comparison of the morphology of spirit-preserved nymphs, working backwards from the last instar, suggests seven or eight; the number is not necessarily constant. In what follows, the instars will be numbered from the adult backwards, i.e. A—1 (final nymphal instar), A—2, etc.

An attempt was made to estimate the activity of the crowded and isolated nymphs by recording the number walking within a period of 5 sec every 2 hr during daylight.* Out of 246 individual sightings of the isolated nymphs over 19 days, only 7 registered walking: and out of 1001 sightings of crowded nymphs, 55 registered walking. The activity of the crowded nymphs was not recorded on an individual basis, so that a comparison between the two groups in terms of the variation between individuals within the groups is not possible. A χ^2 test ignoring this variation would tend to overstate the significance. In fact χ^2 is not significant, i.e. there was no significant difference in the proportion of isolated and crowded nymphs in motion. The respective percentages are 2·8 and 5·5. Even if one were prepared to regard this difference as real, the striking fact is not that the one figure is nearly twice the other, but that in both cases over 94 per cent. of the insects were at rest. This means that 94 per cent. is a conservative estimate (a 5-sec assessment period being used in place of "instantaneous" assessment) of the average proportion of time spent resting by one insect, even in a crowd. In the Phasmatodea the possibility of nocturnal activity must be taken into account. Although no quantitative data are available, the author's general impression is that the laboratory insects were no more active at night than in the daytime and this agrees with observations of Mr. P. Hadlington (personal communication) in the field.

Two of the isolated nymphs, a male and a female, reached the adult stage: the third died in instar A—3, and the fourth only 4 days after the start of the culture, probably in the second instar (A—6). In the crowded culture there were eight deaths during the first 4 days, but the remaining 11 survived for 7 weeks: most of these reached the final instar and several became adult. In the later instars the original wire-gauze cages were exchanged for similar cages of twice the height, so as to enable the nymphs to clear the floor when hanging down in the course of the moult.

At the start of the cultures all the nymphs were more or less uniformly light green. The four isolated ones retained substantially this colouring into the adult stage or until death (see Section III(*b*), "Low-density Phase"). However, about 2 weeks from the start of the cultures, dark areas began to be noticeable on various parts of the body of the crowded nymphs, then in instar A—6. Within 10 days, all but one of these had developed a considerable amount of black. The exception was indistinguishable from the isolated nymphs: it had lost a leg at an early age and was decidedly smaller than its cage-mates. In the later instars all the crowded nymphs except this one lost all trace of their original green colour and attained a striking black and yellow-buff pattern approaching that described in detail below (Section III(*b*), "High-density Phase"). By instar A—2 even the exception had acquired some black areas, principally the abdominal venter, the mesonotum in the region of the spines, the hind coxae, and a mid-dorsal line on the abdomen. Plates 1 and 2 are paintings from life of a male nymph from one of the isolated cultures and one showing

*i.e. each insect was allowed 5 sec in which to display walking activity at each inspection. Ellis (1951) appears to have relied upon "instantaneous" assessments of activity in her work on locusts. However, every such assessment in fact covers a definite, though short, time interval, which could vary systematically in such a way as to affect the accuracy of the conclusions: it seems preferable to standardize this interval. "Walking" is defined as activity leading to a change of location: for the most part it corresponded to the "pottering" of Ellis (1951).

the greatest development of black pigment among those from the crowded culture. The structural features of the isolated specimen were drawn when it was in instar A—2, but by the time the colouring was done it had passed into A—1. All work on the crowded specimen was done in A—2.

Although the numbers reared were small, the result is quite clear-cut. Eleven nymphs reared in a crowd all developed a considerable amount of black pigment and all but one developed a striking black and yellow-buff pattern with no green. Three isolated nymphs remained more or less uniformly green. The only difference in the conditions of culture between the two groups was the number per cage.

Examination of exuviae from the crowded culture showed that the black pigment is located in the exocuticle, all the characteristic areas being glossy black. The non-black areas were straw in the exuviae, the appendages being covered with an extremely fine pile of black hairs. Exuviae of the isolated nymphs were straw to white. Thus the pigments responsible for the yellow-buff and green colours are presumably located in the epidermis.

(*b*) *The Phase Patterns: Description and Rating*

It is clear that the pattern types developed by nymphs of *P. wilkinsoni* do in fact represent kentromorphic phases (see definition of "phase" given by Key 1950, p. 402). There is no evidence of overt gregarious behaviour in dense populations of this phasmatid, so that the terms "*solitaria*", "*gregaria*", and "*transiens*", as used for locusts, are not applicable (see Key 1950). It is proposed, instead, to introduce the terms "low-density phase" and "high-density phase", between which an "intermediate phase" may be recognized.*

The descriptions that follow are based on the most extreme individuals available in material from three different sources: (1) the laboratory cultures described in the preceding section, (2) material collected by the author in the course of field observations in the Jenolan area of New South Wales in December 1954 (see Section III(*c*)), and (3) museum material collected at various times and places by officers of the Forestry Commission of New South Wales and others (see Section III(*d*)). They refer to the pattern in life, preserved material being interpreted in the light of living specimens. It will be noted that the pattern differences characteristic of the nymphs persist to a considerable extent in the adults and that there are sexual differences in the pattern of adults and late nymphs.

Low-density Phase (*extreme*)

Nymph.—In the earlier instars, up to and including A—4, the colour is more or less uniformly light green, except for the rudimentary spines on the mesonotum and those on the ventral carinae of femora II and III, which are black. By the last three instars some differentiation of pattern has become evident, although the general

* These terms are applicable to all animals with kentromorphic phases, irrespective of whether gregariousness is one of the characteristics of the high-density phase or swarms are ever produced. "Intermediate" seems preferable to "transitional" (based on "*transiens*"), since this phase is not necessarily transitional between the two extremes in any dynamic sense, even in locusts, but may be maintained as a steady state at intermediate population densities.

appearance is still that of a uniformly green insect. For instar A—3 this pattern may be described as follows: Antenna, femora and tibiae of all legs, dorsal surface of head and pronotum, and whole of ventral surface, yellowish green. Meso- and metanotum, tegmen-wing rudiments, and dorsal surface of abdomen cephalad, a more bluish green. Abdomen often with a very thin, deep blue-green mid-dorsal line over much of its length. Mesonotal spines yellow, the extreme tips black. Mesosternal tubercles white apically, ringed with black at the base. Metasternal tubercles white, sometimes very narrowly ringed with black. Spines on femora II and III black. Tarsi brown on the distal article.

The pattern of instar A—3 persists with little modification in the remaining instars. However, the ventral surface of the thorax, legs, and often abdomen is more of a buffish green in A—2, tending towards buff on coxae and trochanters II and III and adjacent part of the corresponding femora; this effect is somewhat more pronounced in A—1, especially in the male (Plate 2(*a*)), where a mauvish tinge may also appear on the thoracic sterna, abdominal sternites, and especially the poculum. The same mauve tinge may be present in the male among the spines on the mesonotum. In A—2 the mesonotal spines are yellow to orange, sometimes without black tips in this instar, and in A—1 usually so. The mesosternal tubercles are sometimes wholly black. In A—1 the spines on femora II and III may be black only at the extreme tip, the remainder of the spine being green; in the female the caudal part of the mesonotum, the metanotum, tegmen-wing rudiments, and the cephalic part of the abdominal dorsum often tend to be whitish.

Adult male.—Antenna pale brown with greenish tinge, each article blue-green distad. Eye buff. Head, pronotum, and mesonotum mauvish buff with green tinge, the cephalic margin of the pronotum and the mesonotal spines pale blue-green. Metanotum marked with blue and purple. Abdominal dorsum mainly greenish buff, more mauvish buff on terga IX and X, with a narrow, purplish brown mediolongitudinal line. Mesopleuron pale yellow-brown, the tubercles tipped with blue-green. Metapleuron green; a small area cephalad beneath wing-base black, this narrowly separated from the green by pale blue. Pro-, meso-, and metasternum pale yellow-brown, the tubercles on the mesosternum somewhat darker and on the metasternum greenish. Abdominal venter buff-green, more brown along caudal margin of each sternite, especially mesad; poculum brown-purple. Tegmen pale yellow-green caudad of the concave fold, purplish brown cephalad of the fold, with a narrow white stripe along the cephalic margin (except in the distal quarter) and the knob black. Wing in folded position buffish green dorsad, purplish brown laterad; when spread, orange on the greater part of the pre-anal region normally covered by the tegmen, the rest pale purple, the colour being concentrated mainly in the veins. Legs buffish green, the tarsi pale brown tinged with green, coxa and trochanter III pale brownish purple.

Adult female.—Antenna, eye, head, pronotum and mesonotum, tegmen, wing in folded position, and legs in dorsal and lateral aspect, pale green to greenish straw, except as follows: shoulder of mesonotum sometimes narrowly dull brownish purple or pale purple caudad, this continued as a narrow buff to purplish shoulder stripe, rapidly becoming straw, along tegmen; veins of tegmen and of wing in folded position

straw; genicular lobes of all femora dark brown to black distad. Metanotum pale purple, pale blue around wing-base. Wing, when spread, as in male on the normally concealed areas. Abdominal dorsum very pale blue-green laterad, very pale mauve in mesal region, mid line pale purplish brown broadening a little and becoming dark brown on tergum IX. Meso- and metapleuron green, the latter with black and pale blue areas cephalad as in male. Pro-, meso-, and metasternum green; the rudimentary abdominal sternum I (a narrow, transverse piece of partially unsclerotized integument immediately caudad of the metasternum) orange. Abdominal venter rich velvety green, some of the sterna in the central region with a brown spot mesad on their caudal margins, operculum and cercus green. Legs in ventral aspect green, tinged with brown on coxae and trochanters II and III and adjacent part of femora.

High-density Phase (extreme)

Nymph.—Differences from the low-density phase are first evident in about instar A—6, when dark areas appear on the distal extremities of the femora, tibiae, and tarsi of all legs and among the spines on the cephalic part of the mesonotum. Later these areas become quite black, and the mesonotal one extends until the whole of the dorsal surface from pronotum to abdominal apex (except for the tegmen-wing rudiments) and the dorsal part of the meso- and metapleuron are black, followed by the prosternum and the ventral surface of the abdomen. The rest of the insect remains green.

By instar A—4, the pattern that will characterize the remainder of the nymphal period has been substantially attained. This may be described as follows: Antenna straw to yellow-buff. Head in dorsal and lateral view buff to brown cephalad, near-black caudad of eyes. Pro-, meso-, and metanotum, pleura, and abdominal tergites almost to the apex of tergum X black, with a light mottling of straw on metanotum and abdominal tergum I; cercus and extreme apex of tergum X mainly yellow-buff. Mesonotal spines wholly black. Tegmen-wing rudiments straw. Ventral surface of head and cercus buff suffused with black; remainder of ventral surface black, with a light mottling of buff on the metasternum (especially between coxae III) and very fine and sparse buff stippling on the abdomen. All coxae and trochanters, with the immediately adjacent part of the femora, black. Distal $\frac{1}{3}$ of femora II and III, with their spines, distal $\frac{1}{4}$ of femur I, and distal $\frac{1}{3}$ of all tibiae, along with their extreme base, black. The three distal articles of all tarsi and the distal extremities of the two proximal articles, black. Remainder of all legs, yellow-buff.

Changes that occur in the above pattern in later instars are minor in character and comprise on the whole a diminution rather than a further expansion in the distribution of black pigment, especially in the female. In A—3 the mesonotal spines become yellow to orange, with only the tips black. The metanotum and most of abdominal tergum I is straw, with a mediolongitudinal black line on the latter, and the black colour of the remainder of the abdominal dorsum is broken by a considerable amount of yellow-buff mottling on tergum II and by very fine yellow-buff stippling, decreasing in amount caudad, on terga III–VIII. Small buff areas are present between the coxae on both meso- and metasternum and some tubercles on the latter are buff-tipped. In males of the last two instars (Plates 1(*b*) and 2(*b*) show almost the extreme

condition) the following further changes may be noted: The antenna is buff, becoming almost black distad and in A—1 on the proximal article also. Head black caudad of the eyes and in A—1 wholly black dorsad and laterad, except for a small buff triangular area on the vertex. Mesonotal spines orange in A—2, nearer to orange-brown in A—1, the extreme tips black. Metapleuron with the tubercles yellow-buff. In A—2, and even more in A—1, the surface of the wing rudiment between the raised straw veins is dark brown to black; in A—1 the tegmen, also, is near-black in the distal $\frac{2}{3}$, with the veins yellow-buff and in the proximal $\frac{1}{3}$ yellow-buff lightly streaked with near-black. The black patches dorsad on femora and tibiae II and III occupy only c. $\frac{1}{5}$–$\frac{1}{4}$ of their length in these instars, and those on femur and tibia I less than this; however, in A—1 the whole of tarsi II and III is black and the whole of tarsus I apart from some brown on the proximal article. In A—1, also, tibia III has a black suffusion extending the whole length in the vicinity of the carinae. In A—1 all the non-black areas are rather uniformly yellow-buff to dull chrome yellow, except on the thoracic sterna, where they are buff to light yellow-brown; in A—2 they are lighter, more as in A—3, i.e. nearer straw or tegmen-wing rudiments, metanotum, and abdominal tergum I, **and on the legs paler dorsad than ventrad.**

Females of the last two instars have a somewhat different pattern, characterized chiefly by a less extensive development of black areas in comparison with the male: Antenna straw to buff, slightly infuscate distad. Head caudad of the eyes mottled with near-black and yellow-buff, elsewhere straw to yellow-buff. Pronotum black, with a narrow buff zone along caudal margin. Mesonotum black, except caudad near wing-bases and caudal apex, where straw to cream or whitish. Mesonotal spines yellow-orange to orange, with black tips or in A—1, with some of the tips not black. Tegmen-wing rudiments, metanotum, and abdominal tergum I uniformly straw to cream or near-white faintly tinged with turquoise. Ground colour of abdominal dorsum cream to buff from tergum II to c. V–VII. From the caudal portion of tergum I a black mediolongitudinal line and, on either side, another along the ventro-lateral margin of the tergites extend caudad, widening progressively, until by about tergum V–VII the black colour is predominant, with a buff mottling or stippling which becomes very slight on tergum IX. Tergum X and cerci as in instars A—4 and A—3. Pleura as in male. Thoracic and abdominal sterna as in A—3 and the male of later instars, the operculum and sternum X somewhat mottled black and buff in A—1. Legs with the black areas as in the male of A—2, non-black areas as in the male of the corresponding instar. In the female all black areas are for the most part matt, not glossy as in the male. The difference is most evident on the abdominal venter, which in the female is a very distinctive intense velvety black.

Adult male.—Antenna pale brown, dark brown distad. Eye brown. Head and pronotum somewhat mottled with pale brown, purple-brown, and dark brown. Mesonotum dark brown, paling somewhat caudad, the spines a little paler. Metanotum marked with blue and purple. Abdominal dorsum similar to the late-instar nymph, but dark purple-brown replacing black and the buff spotting a little more marked. Meso- and metapleuron dark brown, the tubercles buff, black and pale blue areas developed on the metapleuron as in low-density phase. Pro- and mesosternum brown, the tubercles on the latter dark brown. Metasternum dark brown to black, slightly

mottled with lighter brown, buff between coxae III, many of the tubercles buff-tipped. Abdominal venter dark purple-brown to black, mottled with buff much as in the late-instar nymph. Tegmen and wing as in low-density phase, but greenish areas more buffish green and purplish areas more dark brown-purple. Legs with all coxae and trochanters and the immediately adjacent part of the femora: the knee areas and tibial apices of legs II and III and to a less degree the knee area of leg I: and parts of the tarsi, especially distad: dark purple-brown: remainder buff.

Adult female.—Antenna pale olive, infuscate distad. Eye straw to buff. Head mottled mauve and mauvish straw. Pronotum mottled with mauvish straw, purple-brown, and black, caudal margin mauve. Mesonotum similar, but a larger proportion dark, especially laterad: spines yellow, extreme apices black. Metanotum marked with blue and mauve. Abdominal dorsum with the ground colour mauvish white, the mauve increasing cephalad, tergum X more bluish white: mid line pale purplish grey, becoming broader and dark brown to black on the last three terga, especially tergum IX: ventrolateral border of terga II–VIII black: additional dark brown to black lateral areas on terga IX and X, the greater part of which is near-black. Cerci black. Meso- and metapleuron black, the latter grading to pale grey dorso-cephalad: tubercles pale yellow. Ventral surface of head and prosternum velvety black, the latter buff between coxae I. Mesosternum velvety black, with the cephalic margin and a small area between coxae II buff; some tubercles straw at the extreme apex. Metasternum velvety black, the tubercles pale yellow; cephalic margin greyish buff and an irregular area between coxae III greenish buff; rudimentary abdominal sternum I orange-buff. Abdominal venter velvety black with small widely scattered buff markings, most extensive on sternum VII: some tubercles cream to pale yellow. Operculum black over a broad mesocephalic area, caudad from which narrowly black mesad, becoming brown caudad: remainder straw-green. Tegmen pale olive-green, the veins straw: shoulder area narrowly purple-brown from tegminal base to a black spot on the knob, thence distad narrowly straw along concave fold almost to apex. Wing in folded position very pale buffish olive-green, veins straw to buff: when spread, as in male on normally concealed areas. Legs with the coxae, trochanters, the immediately adjacent part of the femora, the knees, apices of tibiae, and the whole of the tarsi, black. Femora II and III elsewhere buffish green on dorsal and caudal faces and femur I on dorsal, caudal, and cephalic faces: II and III buff to greenish buff on ventral and cephalic faces and I on ventral face: II and III black along ventro-cephalic carinae, with dark brown bands extending dorsad over part of the cephalic face. Tibiae greenish buff ventrad, buffish green on remaining faces; ventral carinae dark brown.

In addition to the normal type of female, in which the ground colour is green—throughout in the low-density phase, on the tegmen and pre-anal part of the wing only in the high-density phase—a minority have a ground colour which in different individuals ranges from straw-yellow through pinkish buff to salmon. This colour form has already been noted by Froggatt (1905). It shows the same differences between low-density and high-density colour patterns as the normal form. The main features of one such female in the low-density phase (from *Eucalyptus scabra* Dum.-Cours. at Jenolan Caves, December 1954) were recorded in life as follows: Head pale

yellow to cream. Pro- and mesonotum pale yellow to yellowish straw, the shoulder caudad on the latter very pale mauve. Abdominal dorsum buff, with a mauve tinge mesad and along caudal margin of each tergum, cream along ventrolateral margin; mid-dorsal line as in normal form, but especially pale and scarcely widened on tergum IX. Almost the whole venter bright chrome yellow. Meso- and metasternum with the tubercles very narrowly ringed with brown at base, more distinctly so on the mesosternum; rudimentary abdominal sternum I orange. Tegmen and wing in folded position buff, the veins cream; tegmen with pale shoulder lines and black knob as in normal type. Legs pale dull yellow on dorsal, cephalic, and caudal faces, with a greenish tinge on the tibiae, especially of leg I, the tarsi more buffish; on the ventral face, femora II and III bright chrome yellow, leg I and tibiae II and III pale yellow with a greenish tinge, trochanters and bases of femora buff to very pale purplish brown.

No male corresponding to the yellow to salmon female has been recognized with certainty, but a male from the same tree as the female just described had an abnormal amount of red pigment on the venter, the mesosternum and the metasternum between coxae III being dull red to coral and sterna IX–X, especially laterad, orange-red to vermilion. This specimen was probably about half way between the low-density and high-density phases.

The above descriptions of the extremes may be summarized as follows: The low-density phase is almost uniformly green in the nymph and also, as a rule, in the adult female, although a minority of the latter may be straw-yellow to salmon; the adult male is also largely green and lacks any very dark pigment, but is more variegated. The high-density-phase nymph has the greater part of the body black, variegated with yellow-buff, especially on the legs and the dorsum of the prothorax, but without any green; the female has rather less black than the male and tends to be whitish dorsad on the prothorax and cephalic part of the abdomen; the adult has extensive dark areas (black in the female), especially on the venter, but also some green (or straw-yellow to salmon in a minority of females).

These extremes are connected by a continuous series of intermediate patterns, due to intermediate densities (see Section III (c)), in which black pigment occupies an increasing area as one passes from the low-density to the high-density extreme. If such intermediates are arranged in order of increasing area of black and the regions of the body that are black are listed for each, we find that certain specific regions are always the ones affected when the area of black is smallest, while others become affected only when the extreme extension of the black area is approached, i.e. there is an order of responsiveness of different regions to the process of phase transformation. This order, based on nymphs from various localities and dates and from populations of various densities and histories, is set out in Table 1: it may be seen that it differs slightly as between the sexes. Some individuals have patterns indicating a departure in detail from the usual order. Thus one female nymph in instar A −2 has coxae II and III only partly black, although the lateral abdominal line, abdominal venter, and coxa I and knee I are already partly black. Such discrepancies are minor, however, and it appears that individuals moving towards the low-density phase must lose their black in approximately the reverse order, region for region, from that in which they acquire it in the course of the forward transformation.

The sequence of regions in Table 1 has been divided into three sections, of which the first comprises five regions and the third six in both sexes, while the second comprises six regions in the male and four in the female. A numerical "phase pattern rating" of 4 is assigned to individuals in which all the listed regions are black, i.e. to the extreme high-density phase, and of 1 to those showing no black (except on

TABLE 1

ORDER OF BLACKENING OF DIFFERENT BODILY REGIONS IN THE PROCESS OF TRANSFORMATION FROM THE EXTREME LOW-DENSITY PHASE TO THE EXTREME HIGH-DENSITY PHASE IN NYMPHS OF PODACANTHUS WILKINSONI, WITH RATING VALUES ASSIGNED TO DIFFERENT LEVELS OF TRANSFORMATION

Regions bracketed are affected approximately simultaneously. "Part" signifies part only of the area affected in the male of the extreme high-density phase

	Male		Female
	Low-density Extreme (no black, except on spines)		
	1		
increasing area of black	Tarsi Mid-dorsal abdominal line Spinose area of mesonotum Coxae and trochanters II, III Knees II, III	increasing phase rating	Tarsi Mid-dorsal abdominal line Coxae and trochanters II, III Spinose area of mesonotum Knees II, III
	2		
	Abdominal venter (part) Abdominal dorsum (part) Head (part) Pronotum (part) Coxa and trochanter I Knee I		Lateral abdominal line (part) Abdominal venter (part) Coxa and trochanter I Knee I
	3		
	Wing Head Pronotum Abdominal venter Abdominal dorsum Metapleuron		Lateral abdominal line Abdominal venter Pronotum (part) Head (part) Abdominal dorsum (part) Metapleuron
	4		
	High-density Extreme (maximal black)		

the spines of mesonotum and legs), i.e. to the extreme low-density phase. Individuals with all the regions in the first section black, but none of those in the second, are assigned a rating of 2, and individuals with all the regions in the second black, but none of those in the third, a rating of 3 (Table 1). Individuals with only some of the regions of a given section black may be assigned a pro rata rating, approximate to the first decimal, based on the number of regions in the section and the number black in the individual, and incomplete expression of a given character, e.g. coxa I

or metapleuron only partly black, can be dealt with similarly. By this means a continuous rating scale is provided by which the phase level of an individual may be quantitatively expressed. The scale works well on nymphs from instar A—4 onwards. It is not so satisfactory for adults, but can be used to give an approximate rating by omitting "wing" from the list of regions in the male and accepting dark brown in place of black. The order of regions listed in Table 1 is similar to that in which black pigment is first developed in the early instars of dense populations. Further study may show that the rating scale is applicable to these instars also. The low-density-phase individual illustrated in Plates 1 and 2 would be given a rating of about 1·3 and the high-density-phase individual about 4·0.

If an adequate sample of nymphs of instar A—4 or older is taken from any population homogeneous with respect to its density history, the females are seen to have less black than the males. This is partly because even the extreme high-density pattern comprises less black in the female, but partly also because on the average the females have advanced less far towards the extreme pattern, and thus have a lower phase rating.

It is of interest to note that the order of responsiveness of different regions has its parallel in an order of responsiveness of parts of regions. Thus the first part of the coxa-trochanter region to go black is always a small boss on the ventral surface of the trochanter, and the first part of the abdominal venter is the mid point of the caudal margin of each sternite.

(c) *Field Observations on Density and Phase Pattern*

In December 1954 a visit was paid to the Jenolan–Hartley area, N.S.W., in order to study in the field the relation between phase pattern and population density. This area is one of those liable to severe outbreaks of *P. wilkinsoni* (Richards 1952), but 1954–55 was not an outbreak year. Nymphs in the later instars were found at five places: *c.* 4 miles NW. of Hartley, near Hassan's Walls; 1 mile N. of Hampton; *c.* 1 mile and *c.* 2 miles W. of Jenolan Caves, on the Oberon road; and a few hundred yards below the Caves, in the valley of the Jenolan River. At the third and fourth of these, *D. violescens* nymphs were present in addition to *Podacanthus*.

The insects were sitting on the outer foliage of young eucalypt saplings of a variety of species and the lower branches of older saplings. Doubtless they also occurred higher up on the latter, as well as on mature trees. The leaves of eucalypts are characteristically restricted to the distal branchlets, and it was found that the phasmatids occurred almost exclusively on the distal 15 in. of the latter. This length of branchlet, including all side-shoots of less than 15 in. originating within it, thus constitutes a convenient sampling unit for estimating the density of the insects. Seven saplings 4–10 ft high, bearing nymphs of *P. wilkinsoni* at various densities, were selected for sampling, five at the Hassan's Walls locality and one at each of two of the Jenolan localities; in addition, two low boughs of a larger sapling were sampled separately at the third Jenolan locality, making nine sampling sites in all. All the branchlets of a given sapling or bough were examined systematically (except at one site, where on account of the high density only part of the sapling was searched) and the number of nymphs of both species present per distal 15 in. recorded. In no

instance was the number of branchlets less than 14: usually it was more than 30. The nymphs were removed as they were counted, for subsequent pattern rating of *P. wilkinsoni*. The whole operation had to be carried out with a minimum of disturbance; otherwise the insects would drop to the ground before they could be located on the plant.

The distribution of the insects over the saplings appeared to be random in some instances, but in others was significantly clumped. Thus one sapling at Hassan's Walls had nine nymphs on one branchlet, one on another, and none on the remaining 49 branchlets examined. Such a distribution could be brought about in either of

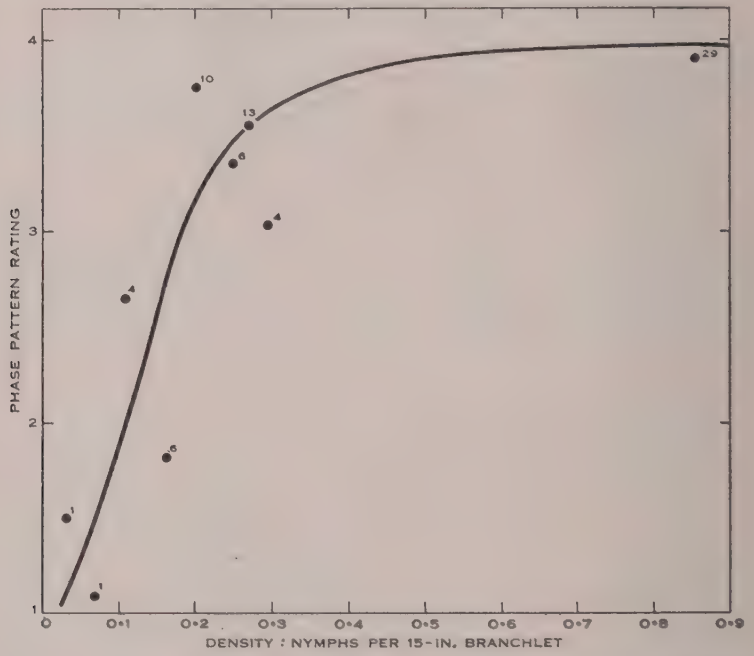

Fig. 1.—Relation between mean phase pattern rating of *Podacanthus wilkinsoni* nymphs and mean density of nymphs of that species (plus some *Didymuria violescens*) per 15-in. branchlet, at nine sampling sites in the Jenolan area, N.S.W., in December 1954. Numerals indicate number of phasmatids contributing to each point.

two ways or by a combination of both: the insects could accumulate quite independently on branchlets having certain preferred characteristics; or they could aggregate, by virtue of some degree of gregariousness, on any branchlet on which initially two or three had come together by chance. It was clear that they occurred more abundantly on the terminal than on the lateral branchlets: these are in general both the highest and the most actively growing, and could well be preferred on both grounds. No overt gregarious behaviour could be discerned.

It is probable that the observed groupings, however they may be produced, are transient. They do not, therefore, detract, to the extent that might be supposed,

from the significance of a mean figure of nymphs per branchlet for the sapling. Although nymphs move about quite considerably on a small sapling, they probably do not leave it. On the other hand, accessions of nymphs that fall from overhanging branches of trees may take place. As a first approximation, use of the mean density of the two species combined per 15-in. branchlet as a measure of population density for correlation with phase status would seem to be reasonable, although the effective density may be considerably higher than this on account of the frequently clumped distribution.

The nymphs collected were brought to Canberra, their phase pattern rated by the rating scale appropriate to their sex, and a mean rating calculated, for the sexes combined, for each sampling site. Figure 1 is a plot of these mean ratings against the corresponding densities per branchlet. Although the data are inadequate for any precise conclusions to be drawn, it would appear that densities of less than 0·1 were required to produce a phase rating below 1·5. The rating rises steeply with increasing density above that value, begins to flatten at a density of 0·3, and by a density of 0·8 is making an asymptotic approach to the limiting rating of 4·0. The very great effect of density change, within a range representing only a small fraction of the full range that must occur in the field between very sparse populations and mass outbreaks, is remarkable.

(*d*) *Phase Pattern in Museum Material*

A quantity of museum material of *P. wilkinsoni*, most of it preserved dry, but some in spirit, was available for study. The bulk of this had been collected by officers of the Forestry Commission of New South Wales, which has kindly made available notes containing subjective assessments of the abundance of the insects at most of the localities and dates concerned. Where such notes were not available, there was usually information as to whether the material had been collected in a plague year and therefore probably from rather dense populations, or in a non-plague year and therefore probably from rather sparse populations. The patterns of this material, comprising almost 100 specimens of which about a third were nymphs, were rated. A mean rating was calculated for each series of specimens (i.e. those from a single locality and date) and the series means grouped according to density, as shown in Table 2. The assessments listed in the second column comprise the range of terms actually used by the various collectors. Data obtained on the author's visit to the Jenolan–Hartley area, referring to 10 specimens for which no precise estimate of population density was made, have been incorporated in Table 2 on the same basis as the museum material.

The last column of Table 2 gives the mean phase rating (mean of the series means) for each density class and for unclassified material from plague and non-plague years. The values for classes I–III are not significantly different, but all are significantly less than classes IV and V, which are not significantly different from each other. The values for plague and non-plague years are also significantly different. Thus the results of a study of museum material from a number of different localities and dates support those of the more intensive and precise analysis of material from a single locality and month.

(e) *Morphometric Phase Differences*

Inspection of the museum material suggested that the length of femur III was in general less in adults from dense populations than in those from sparse. In view of the findings of Dirsh (1951, 1953) and of Misra, Nair, and Roonwal (1952) in regard

TABLE 2

PHASE PATTERN RATINGS OF MUSEUM MATERIAL OF PODACANTHUS WILKINSONI (ADULTS AND NYMPHS) COLLECTED AT VARIOUS LOCALITIES AND ON VARIOUS DATES AND CLASSIFIED ON THE COLLECTORS' SUBJECTIVE ASSESSMENTS OF ABUNDANCE

Ratings are the unweighted means of series means

Density Class	Abundance Assessments by Collectors	No. of Series	No. of Specimens	Mean Rating
I	Only one. Very light. Very scarce	7	7	1·23
II	Light. Few. Scarce. Three only	6	13	1·79
III	Medium number. Fairly plentiful	3	10	1·81
IV	Plentiful. Exceptionally plentiful. Fairly heavy. Heavy in patches	7	26	3·31
V	Heavy	3	28	3·12
Unclassified: non-plague year		3	5	1·40
Unclassified: plague year		8	15	3·53

to the value of the ratio F/C (length of femur III greatest width of head) for distinguishing the kentromorphic phases of locusts, this ratio was determined for all measurable specimens. The dimension F was taken along the dorsoexternal carina of the femur, from its articulation with the trochanter to its distal extremity (Fig. 2);

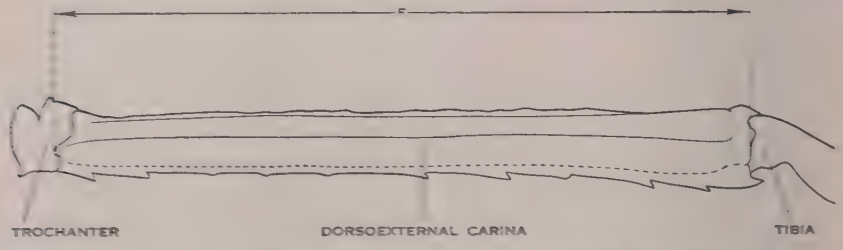

Fig. 2.—Left femur III of a female *Podacanthus wilkinsoni* in dorsoexternal aspect, showing the dimension *F*.

the measurement was made with dividers, under a binocular microscope, and a scale graduated in $\frac{1}{2}$ mm. The dimension C was taken by means of an eyepiece grid. The error of measurement probably never exceeded 0·1 mm in either case. Care was taken not to measure regenerated legs, which are commonly found in Phasmatidae.

Series means of F, C, and F/C were calculated for the sexes separately and grouped in density classes, as shown in Table 3. These classes are the same as those of Table 2, except that classes II and III, which scarcely differed in their mean

TABLE 3

MORPHOMETRIC DATA FOR MUSEUM MATERIAL OF PODACANTHUS WILKINSONI (ADULTS) COLLECTED AT VARIOUS LOCALITIES AND ON VARIOUS DATES AND CLASSIFIED IN DENSITY CLASSES AS IN TABLE 2

F = length of femur III, *C* = greatest width of head. Values are the unweighted means of series means

Density Class	Males					Females					C_{φ}/C_{δ}
	No. of Series	No. of Specimens	*F* (mm)	*C* (mm)	*F*/*C*	No. of Series	No. of Specimens	*F* (mm)	*C* (mm)	*F*/*C*	
I	2	2	20·15	4·08	4·95	2	2	19·20	5·12	3·76	1·25
II + III	2	2	20·25	4·36	4·65	3	4	19·78	5·09	3·89	1·17
IV	3	6	19·03	4·42	4·30	2	6	17·85	4·97	3·59	1·12
V	2	4	18·06	4·47	4·04	3	24	17·21	4·85	3·55	1·09
Non-plague year	1	1	20·30	4·40	4·61	2	3	18·48	4·85	3·82	
Plague year	4	4	17·99	4·35	4·14	2	3	18·18	5·08	3·58	1·17

pattern rating (Table 2), have been combined. In addition to the class means, the sexual dimorphism ratio with respect to head width ($C♀/C♂$) has been calculated on the basis of the class means for C. The exclusion of nymphs and damaged adults from the morphometric study, together with the need to treat males and females separately, has greatly reduced the number of specimens contributing to the respective means. Nevertheless, an analysis of variance indicates that the variation among the means for classes I–V is significant for C and F/C in the males ($P<0{\cdot}05$) and for F in the females ($P<0{\cdot}01$). This analysis did not take account of the trends, so that the criteria for significance must be regarded as conservative.

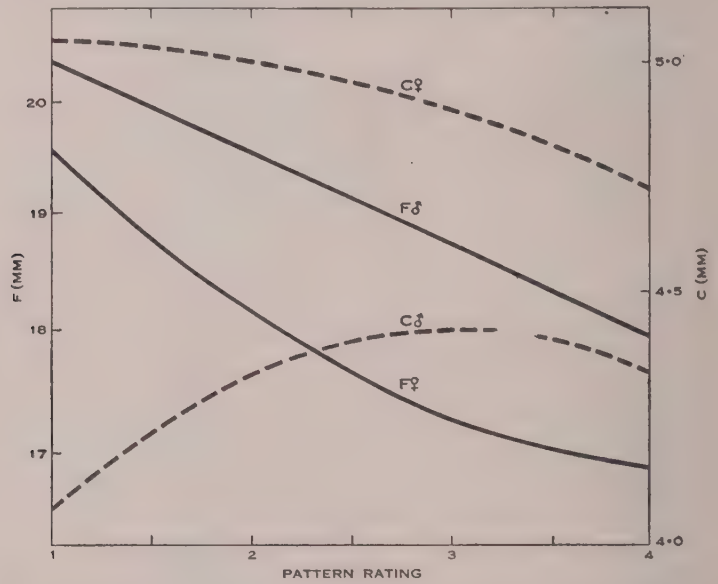

Fig. 3.—Regressions of length of femur III (F) and width of head (C) of museum material of *Podacanthus wilkinsoni* (adults) on pattern rating. All characters on the same logarithmic scale.

If we disregard an irregularity involving some of the measures in class II + III, the general picture presented by Table 3 is as follows: F decreases markedly in both sexes with increasing density; C decreases in the female, but increases in the male; F/C decreases in both sexes, but much more markedly in the male; and $C♀/C♂$ decreases steadily. Class for class, F is larger in the male sex and C in the female: the ratio F/C is in consequence considerably larger in the male. It is likely that a study of further material would show that most of these differences are real. It may be noted that with increasing density the ratio F/C varies in the same direction in *P. wilkinsoni* as in the locusts that have been studied. The sexual dimorphism ratio in locusts is normally based on tegmen length; it is lower in dense populations. However, it has been shown (Misra, Nair, and Roonwal 1952; Key 1954) that, in certain species at least, the ratio based on head width behaves in the same way; so that, with respect to sexual dimorphism also, *P. wilkinsoni* agrees with the locusts.

In view of the subjective character of the density classes, it is desirable to examine the relation between the morphometric characters and pattern rating; the latter has been shown to be closely correlated with density and might well be a more reliable indicator of the effective density operating on an individual than are the subjective assessments available. Figures 3 and 4 represent quadratic regression lines of each of the morphometric characters (on a logarithmic scale) on pattern rating. The same logarithmic scale is used for all characters, so that the slopes of the lines afford direct comparisons of proportional change. Figure 4 includes in addition

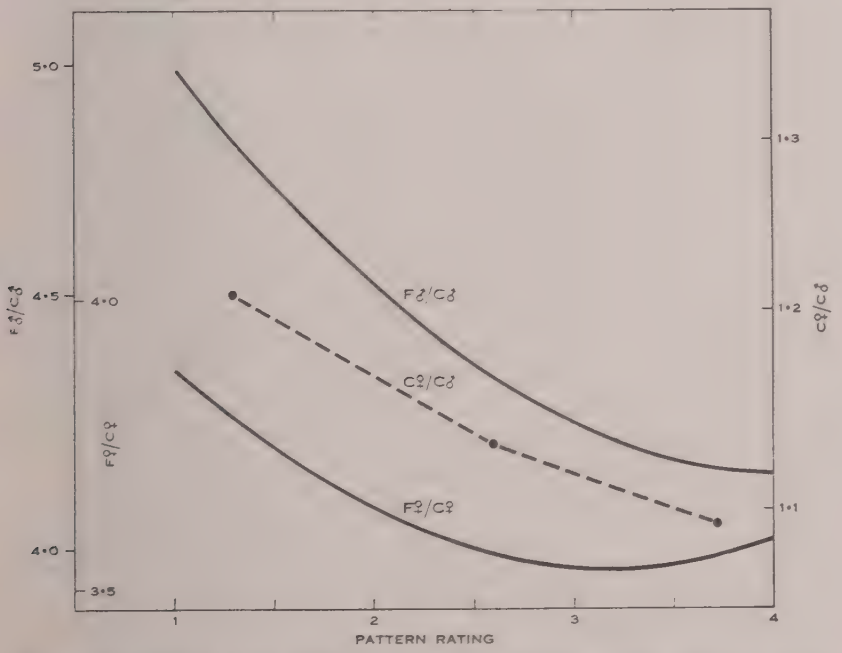

Fig. 4.—Regressions of the ratio length of femur III width of head (*F C*, on pattern rating in museum material of *Podacanthus wilkinsoni* (adults), together with a plot of $C♀ C♂$ based on the mean values of *C* and of pattern rating in three pattern rating ranges. All characters on the same logarithmic scale.

a plot of $C♀ C♂$ based on the mean values for *C* corresponding to three pattern rating ranges. The linear component of each regression is significant except that for $C♂$, the *P* values being as follows: $F♂$, $< 0{\cdot}05$; $F♂ C♂$, $< 0{\cdot}01$; $F♀$, $< 0{\cdot}001$; $C♀$, $< 0{\cdot}01$; $F♀ C♀$, $< 0{\cdot}001$. The quadratic component is significant only for $F♀ C♀$ ($P < 0{\cdot}05$). The plot of $C♀ C♂$ is also significant, the difference between the first and second points giving $P < 0{\cdot}05$ and the first and third points $P < 0{\cdot}001$.

The indications of the regression lines support those of Table 3, at a considerably higher level of significance, except with respect to $C♂$. It is not clear what interpretation should be placed upon the increase in $F♀ C♀$ at the highest pattern ratings.

(*f*) *Characterization of the Phases*

If the terms "high-density", "low-density", and "intermediate" phase are to be useful, each should cover a range in the degree of development of the phase characters, i.e. the first two should not be confined to those individuals that conform in every respect to the descriptions of the two extreme conditions given in Section III(*b*), leaving the great majority of individuals in the "intermediate" phase (cf. Key 1950). While it is no doubt premature to attempt to define the phases rigidly, some indication of what might constitute suitable ranges needs to be given.

As in locusts, it seems best for practical purposes to select the most appropriate phase character for the species concerned and to base the phase definitions on that character alone. In *P. wilkinsoni* this would seem to be the colour pattern, for a pattern rating can be obtained for both nymphs and adults and does not depend on particular organs being undamaged. It is proposed that individuals with a pattern rating $< 1{\cdot}5$ be regarded as in the low-density phase, those with a value $> 3{\cdot}0$ in the high-density phase, and those with values from 1·5–3·0 in the intermediate phase. The following information applies to the two critical values of 1·5 and 3·0: At 1·5 dark areas are very limited in number and extent (Table 1). On the basis of museum material, this rating is characteristic of populations that may be designated "light" to "very light" (Table 2). The Jenolan observations found it associated with densities of about 0·07 nymphs per 15-in. branchlet (Fig. 1). The corresponding morphometric values may be determined from Figures 3 and 4; e.g. that for $F♀$, perhaps the most sensitive morphometric character, is about 18·8 mm. At 3·0 the black areas are extensive. In museum material this rating was characteristic of individuals designated "plentiful". At Jenolan it corresponded to densities of about 0·18 nymphs per branchlet. The corresponding value for $F♀$ is about 17·3 mm.

In view of the greater development of the pattern in the female adult than in the male and the greater ease of rating in that sex, it might be advisable to base determinations of the phase status of populations on females only, wherever the number of individuals available permits this.

IV. Didymuria violescens

Two batches of first- and second-instar nymphs of *D. violescens* were used to set up crowded and isolated cultures, which ran concurrently with those of *P. wilkinsoni* described in Section III(*a*). The cages and their location and the methods of handling the insects were the same as for the latter species. The first batch comprised two nymphs received from Jenolan in October 1954, together with hatchlings from eggs collected earlier in Bago State Forest, near Batlow, N.S.W. The whole of this batch was used to constitute a crowded culture, which originally comprised eight nymphs but was built up to 13 during the first fortnight by the addition of further hatchlings from the Bago eggs. The second batch comprised several hundred nymphs received from the Bago State Forest in November 1954. It was used to set up 12 cultures of one nymph each, two cultures of 25 nymphs each, and two of 50 each. Excess material was kept in a large cage and used to supplement the crowded cultures when deaths occurred.

Mortality in the crowd of 13 was at first lower than in the crowded culture of *P. wilkinsoni*, but it increased considerably after the first month and only four individuals reached the adult stage. In the cultures of 25 and 50 mortality was very much higher; by half way through the nymphal stage, these were probably comprised very largely of replacements from the large stock cage, in which, however, the density was also quite high. The four cultures together yielded only 10 adults. Of the 12 nymphs kept isolated, five became adult.

The insects in all the isolated cultures remained more or less uniformly green throughout the nymphal period and showed no trace of darkening. In the crowd of 13, some began to develop dark areas at about the same time as, or shortly after, the similar development in the crowd of *P. wilkinsoni*, i.e. in the third week from the start of the culture. However, the development proceeded more slowly and did not reach the same extreme end-point; several of the nymphs were only slightly affected. In the crowds of 25 and 50 darkening was less than in the crowd of 13; nevertheless, the early stages were observable on a number of individuals in these cultures also. Three weeks after the start, a male nymph in one of the middle instars had the following areas more or less infuscate: the knees, tarsi, and distal portions of the tibiae of all legs, especially the second and third pair; the lateral fringe of meso- and metanotum and of the tegmen-wing rudiments; the distal third of the wing rudiments; and a small spot in the laterocaudal angle of abdominal terga I–VII.

It is clear that the pattern differences between the isolated and crowded nymphs of *D. violescens* are phase differences, similar to those occurring in *P. wilkinsoni* but less marked. The greatest development of what we may again call the high-density phase was attained in a male in instar A—1 from the crowd of 13, illustrated in Plate 3.

In February 1957 some 40 living specimens of both sexes, mostly in instar A—1, but a few in A—2, were received from the Bago State Forest, where an outbreak of *Didymuria* was in progress. They had been collected from the densest and "darkest" patches of infestation. Examination of these specimens showed that most of the males had a more extreme high-density pattern than the laboratory-reared male illustrated on Plate 3. Some of the females also showed a highly developed pattern, which differed somewhat from that of the males. The following descriptions are based on this material.

High-density Phase (extreme)

Instar A—1, male.—Antenna olive-buff, smoky ventrad and on distal and proximal 2–3 articles. Head faintly olivish dark brown. Pronotum dark purple-brown passing to black caudad. Mesonotum black, the tubercles cream laterad, black mesad. Metanotum dark purple-brown. Abdominal terga very dark purple-brown, all except VIII–X becoming black along caudal margin. Cercus dark purple-brown. Tegmen-wing rudiments purple-brown, their lateral (morphologically cephalic, or costal) margins black; tegmen with a straw line inside the black; wing with almost all the raised veins black throughout their length, whole of apex black. Mesopleuron yellow; metapleuron yellow to orange; both bordered dorsad with black; a thin yellow to buff line continued thence caudad along lateral margins of abdominal terga II and III and much less distinctly on other terga. Prosternum and cervical region

brown. Meso- and metasternum black, the tubercles sulphur yellow. Abdominal venter very dark purple-brown to black, sterna II–VI with a small, slightly longitudinal, buff fleck. Legs with the femora mainly faintly greenish buff dorsad (femur I more buff), buff to brown ventrad, knees of all legs and base of femora II and III very dark purple-brown to black; tibiae buff to brown, heavily infuscate with very dark purple-brown to black, especially distad and along carinae; tarsi dark purple-brown; coxae and trochanters dark purple-brown, those of leg I the palest; all spines black.

Instar A–1, female.—Antenna greenish straw, buff to brown on proximal 2–3 articles. Head pale olive-buff, darker cephalad, becoming dark brown laterad. Pronotum pale olive-buff, with a purplish tinge caudad. Mesonotum pale mauvish grey dorsad, darkening to black along ventral margin, mid line narrowly pale purplish pink, the tubercles white. Metanotum and abdominal dorsum dull greenish and purplish straw, a little darker and less greenish caudad on abdomen, mid line purplish pink, narrowing caudad. Cercus purplish straw. Tegmen-wing rudiments greenish straw, with a purplish tinge on some veins and on the cephalic margins. Mesopleuron yellow bordered with black dorsad; metapleuron orange; a purplish line continued thence caudad along lateral margins of tergum II and less distinctly of other terga. Prosternum, cervical region, and mesosternum cephalad, brown. Remainder of mesosternum and metasternum sepia, each becoming olive caudad, the tubercles sulphur yellow. Abdominal venter as in male but more matt; operculum very dark purple-brown cephalad and along mid line, elsewhere dull olive. Legs as in male, but femora more yellow-brown ventrad and coxa and trochanter I brown.

Instar A–2.—The darkest of the few males in this instar were closely similar to those in A–1, but some areas were a little less dark (head, pronotum, tegmen-wing rudiments, caudal margins of abdominal terga, prosternum, abdominal sternum X, coxae and trochanter I and II, tibiae). The differences can probably be accounted for on the assumption that no specimen as extreme as those in A–1 occurred in the small sample of A–2. The three A–2 females were all far from extreme.

Exuviae of the high-density-phase nymphs were black on those parts corresponding to the black areas of the nymphs and had more diffuse black pigment over the purple-brown areas. Some purple pigment was also present over the purple-brown and purplish areas. The pale areas were straw on the exuviae.

Although in their broad effect the yellowish and near-black patterns of the high-density phase are similar in the nymphs of *D. violescens* and *P. wilkinsoni*, a comparison of the respective figures (Plates 1, 2, and 3) and descriptions will show that in a number of points of detail they are rather different, the one being even the reverse of the other. Thus in *Podacanthus* the mesonotum darkens first in the central region, not laterad as in *Didymuria*, and the abdomen lacks the pale mediolongitudinal line of the latter. The thoracic pleura are black in *Podacanthus*, yellow to orange in *Didymuria*. The wing rudiments have the raised veins pale in *Podacanthus* and the intervening areas dark, whereas in *Didymuria* this relation is reversed. The legs of *Didymuria* are more olivish and less yellow than in *Podacanthus* and the dark areas less sharply demarcated. The two species agree in having the female high-density pattern less dark than the male, and particularly in having the metanotum, tegmen-wing rudiments, and much of the abdominal dorsum very pale.

The Bago material shows that *Didymuria* agrees with *Podacanthus* also in the fact that the females in a field population are on the average less advanced towards the high-density phase than the males. All the males were close to the extreme pattern, whereas the females showed all stages between the latter and mainly green individuals with quite limited areas of black. As in *Podacanthus*, different regions of the body are affected in a definite order in the course of the transformation from the low- to the high-density phase. This order is approximately as follows: The first regions to darken are the rudimentary abdominal sternum 1, the ventral margins of the mesonotum, and the meso- and metapleura dorsad. These are followed in turn by the areas immediately surrounding the mesocaudal flecks on the abdominal sterna, the knees and tarsi of legs II and III, and the extreme lateral margins of the abdominal terga (lateral line), especially in the laterocaudal corners; the knee and tarsus of leg I; the coxa and trochanter of leg III and, in the male, the abdominal dorsum and the mesonotum mesad; the early changes on the head, pronotum, and venter of the male; and the whole venter (completion of darkening) in both sexes. Finally, in the male the last few abdominal segments and the cercus darken, the last traces of green disappear from the tegmen-wing rudiments, and the coxae and trochanters of legs I and II darken. It is clear that a rating scale, similar to that used for *Podacanthus*, could be developed to indicate quantitatively the phase level of nymphs of this species.

The phase pattern of the nymphs persists to some extent into the adult stage, as may be seen by comparing particularly the adults reared from the 1957 Bago nymphs with museum adults collected from sparse populations. The male of the extreme high-density phase has the pronotum, mesonotum, and abdominal dorsum purplish brown, except the mesonotal tubercles and the mid line of the abdomen, which are straw to buff; thoracic pleura cream to buff ventrad; undersurface brown to dark brown, the thoracic tubercles buff to black. In the extreme low-density phase all these regions are uniformly fawn to green, except, in form 1, the more cephalic abdominal terga. The extreme high-density-phase female has the rudimentary abdominal sternum 1, the thoracic pleura dorsad, and the lateral margins of the mesonotum and abdominal terga black; head laterad behind the eyes and greater part of legs ventrad, including coxa and trochanter III, grey; venter generally grey-green; pale stripe ventrad on thoracic pleura pinkish. In the extreme low-density phase there are no black or grey areas. Complications connected with the two forms here included under *D. violescens* (Section II) make it inadvisable to attempt a more detailed description of the patterns, or to undertake an analysis of the phase status of the museum material available. Information on the field density of this material is also inadequate for the purpose. It is of interest to note that, of the experimental material that became adult, a male from the crowd of 13, as well as one reared in isolation, have the characters of form 1. The other males are intermediate between the two forms and only one, from a crowd, seems to be nearer form 2 than form 1. The culture material strengthens the view that the two forms are conspecific, but seems to exclude the possibility of their being a kentromorphic manifestation.

When all the adequately preserved adult females from the cultures were classified into crowded and isolated and their values for *F*, *C*, and *F C* determined as for *P. wilkinsoni*, striking morphometric phase differences, displaying the same trends

seen in field material of the latter species, were immediately apparent. The relevant figures are given in Table 4. It may be seen that the mean values for *F* and *F C* are much lower in the crowded material; there is even no overlap between the ranges for isolated and crowded and the difference is, of course, significant. The mean for *C* is lower in the crowded material, but the difference is not significant. The number of males reared to maturity in the laboratory cultures was too small for analysis.

TABLE 4

MORPHOMETRIC DATA FOR ADULT FEMALES OF DIDYMURIA VIOLESCENS REARED FROM THE EARLY INSTARS IN ISOLATION AND IN CROWDS

F = length of femur III, *C* = greatest width of head. Mean values given in parenthesis

Density	No. of Specimens	*F* (mm)	*C* (mm)	*F/C*
Isolated	4	18·4–20·5 (19·40)	3·6–3·7 (3·66)	5·11–5·57 (5·30)
Crowded	6	13·5–16·7 (15·23)	3·2–3·7 (3·50)	3·86–4·81 (4·36)

V. CTENOMORPHODES TESSULATA

In late September 1956 a batch of 26 first-instar nymphs of *C. tessulata* was received from the Kempsey district, N.S.W. These were used to set up 13 cultures, one comprising 14 nymphs and the remainder one nymph each. The cages, their location, and the methods of handling the insects were essentially as described for the two species already dealt with. However, during the early instars each gauze cage was covered with an inverted plastic jar to keep up the humidity and the cage and its contents were sprayed with water every second day by means of a small atomizer. From about instar IV the plastic covers were discarded, but the spraying continued. Since in the Kempsey district the young nymphs were very abundant on *Acacia floribunda* (Vent.) Willd.,* the cultures were fed a locally available species of *Acacia*, *A. mollissima* Willd.,* in addition to *Eucalyptus dives*; the nymphs appeared to eat more of the acacia than of the eucalypt in all instars.

The moults were followed with care in the isolated nymphs and diagnostic characters found for each instar. These enabled the instars to be determined in the crowded culture. All the males passed through six nymphal instars and the females through seven.

During the first 3 weeks, deaths in the crowded culture reduced the number of nymphs from 14 to 10. Six further deaths during the following week were replaced by transferring isolated third-instar nymphs to the crowd, but mortality continued and only five reached the adult stage, of which the majority had probably lived in isolation until instar III. The mortality was apparently due largely to cannibalism. Dead individuals, usually freshly moulted, were found to have portions of their legs chewed and often there were detached legs, also chewed. Amongst the isolated nymphs mortality was low. Up to instar III only one had died, and only one other death occurred among those remaining after transfer of the six instar III to the crowd.

* Determinations by M. Gray, Division of Plant Industry, C.S.I.R.O.

The colour pattern of the isolated nymphs proved to be more complicated than in the species already dealt with. In the early instars, restricted black or blackish areas were present on a green background. As development proceeded these areas became mottled blackish and cream. In the males they remained restricted and the greater part of the body remained green at least to the end of instar V. In the females, however, they expanded and at the same time the green of the general body surface became replaced by olive and finally by brown to dark grey-brown, so that by instar V, and more especially thereafter, the insect was mainly brown to grey, intricately mottled with darker shades and with cream. For the first 8 weeks no differences suggestive of a kentromorphic effect could be detected between the isolated and crowded nymphs: indeed, the latter showed, if anything, a slighter development of blackish areas than the former. However, by the beginning of the ninth week, when the insects were just entering instar VI, one male in the crowd was noticed to have a rather distinct yellow banding on the legs. Within the next fortnight all the crowded individuals developed, in varying degrees, a mottled pattern of jet black, yellow, and white, superimposed on the ground colour characteristic of isolated nymphs of the same instar and sex. This pattern was quite different from anything seen among the isolated nymphs. At this stage the crowd comprised seven individuals and the isolated cultures four. Once again, the difference must be interpreted as a kentromorphic manifestation.

The following descriptions give details of the changes in the pattern of the isolated nymphs as they developed, the sexual differences in that pattern, and the extreme pattern developed by the crowded nymphs in instar VI. The last can be regarded, in the case of the female, as a close approximation to the extreme development of the high-density phase. Late-instar spirit material, collected by officers of the Forestry Commission of New South Wales in the Tanban State Forest in December 1956, includes a few females in instar VI with a pattern similar to that described, but with the pale areas of the mesonotum cream to yellow instead of mauvish white; these are presumably slightly more extreme. On the other hand, the male described is clearly not in the extreme condition, for the spirit material includes one sixth instar of that sex with a pattern essentially similar to the extreme female (i.e. with much more black, especially on head and thorax, and with the lateral mesonotal tubercles yellow in the centre). It is uncertain whether the pattern of the isolated nymphs represents the extreme low-density phase. Four pinned female nymphs in instars VI and VII, from as many different localities in Queensland, have been examined which are almost uniformly green, with none of the brown to blackish markings of the isolated laboratory females. This may be merely a geographic racial difference. However, the nymphs used in the cultures had hatched in an area where a dense population had been present in the preceding season, and there may have been some carry-over of parental phase characters, as has been reported for locusts by Faure (1932) and many later workers.

Instar I.—Dorsal surface green, paler mesad than laterad, the head a somewhat yellower green than the rest. Mesonotum with a number of small white spots (incipient tubercles) laterad. Abdominal terga II–VIII with a pale fleck in each latero-cephalic corner. Ventral surface more uniformly pale green, yellowish adjacent to

coxae II and III. Legs green, yellowish on coxae and base of tibiae; distal part of tibiae, 3 distal articles of tarsi, and distal part of second article, dark brown; some brownish marbling elsewhere on femora and tibiae, especially of leg I.

Instar III.—Male: The paler dorsomedian region of instar I a more definite greenish white stripe. Head and pronotum lined with green and greenish white. Mesonotum laterad, more narrowly on metanotum, and immediately around laterocephalic pale flecks on more cephalic abdominal terga, dark grey-green; the dorsal spines of mesonotum black, lateral tubercles white. Tegmen-wing rudiments green. Meso- and metasternum mostly dark grey-green, abdominal venter uniformly dark green. Prosternum between coxae I, mesosternum cephalad and between coxae II, and metasternum between coxae III, blackish. A near-white lateral line along thoracic pleura and continued along lateral margins of abdominal terga and sterna. Legs with brownish marbling scarcely evident; tibiae not yellowish proximad; tarsi and distal part of tibiae less dark. Otherwise as instar I. *Female*: Dorsomedian stripe as in male. Head and pronotum lined with greyish green and greenish white. Meso- and metanotum laterad, with tegmen-wing rudiments, dark greenish grey; dorsal spines of mesonotum black ringed with white, lateral tubercles white. Abdominal dorsum grey-green on each side of mediolongitudinal stripe, green more laterad; laterocephalic flecks fringed with black, especially on more cephalic segments. Ventral surface mainly dark greyish green, the grey most pronounced on the thoracic sterna. The following areas black, finely mottled with cream: ventral surface of head, prosternum, mesosternum cephalad and between coxae II, metasternum between coxae III, caudad on each abdominal sternum and less extensively cephalad, and most of sterna IX and X. A near-white lateral line as in male, more buffish on thorax. Legs heavily mottled with grey-brown, especially ventrad; coxae black mottled with cream.

Instar VI, isolated.—Male: Head and pronotum pale olive-green, finely lined longitudinally with cream. Mesonotum grey-olive, irregularly lined and dotted with cream and with a fine cream median line. Metanotum and tegmen rudiments pale olive-green, the former with a broad cream median stripe. Wing rudiments pale olive, flecked with cream, a few of the raised veins dark brown distad. Abdominal dorsum pale olive with a cream median stripe, which is nearer white cephalad on each tergum; laterocephalic flecks white, pointed caudad, about $\frac{1}{4}$ the length of the tergum, bounded by black, especially mesad, absent from terga VIII–X. Ventral surface mainly dark olive-green, areas mottled with brown to black and cream as in instar III. Legs indefinitely mottled with olive-green and greenish buff, especially along carinae. *Female*: Dorsal surface brown to dark grey-brown, finely stippled and lined with cream. Abdomen with laterocephalic flecks less sharply defined than in earlier instars, the general appearance of the insect more uniformly brown. Ventral surface more grey-brown and less brown than dorsal and more mottled with cream, the dark mottled areas of the male and of earlier instars little differentiated, because the whole ventral surface is of this character. Legs mottled essentially as the body.

Instar VI, crowded.—Male: Head and pronotum as in isolated male, but the pronotum with fine black lining in the sulci. Mesonotum black laterad; white dorsomesad, on the lateral tubercles, and on the basal portion of the dorsal spines; apical portion of the last black. Metanotum and tegmen rudiments pale green, the former

with a cream median stripe. Wing rudiments pale green, the raised veins flecked with black along their whole length: cephalic (costal) margin mottled with black. Abdominal dorsum pale greyish green, laterocephalic flecks and their black edging as in isolated male; tergum VII with a broad black longitudinal shoulder stripe on each side, pointed caudad and not quite reaching caudal margin (apparently derived from the black edging of the laterocephalic fleck, which latter is reduced to a minute white dot). Ventral surface dark greyish green, the dark mottled areas as in instar III. Thoracic pleura mainly white, the mesopleuron invaded at intervals by black from above, the metapleuron black dorsad. Pleural region of abdomen (on terga) chrome yellow. Legs conspicuously mottled with black, chrome yellow, and green, especially legs II and III and along the lamellate ventroexternal and ventrointernal carinae of the femora, where patches of black and chrome yellow alternate some 7–8 times; the green colour developed mainly on the flat surfaces between the carinae, opposite the black sections of the latter; femora with more black in the genicular region than elsewhere; tarsi mainly black in distal half. *Female*: Head and pronotum streaked with black and mauvish white. Mesonotum black laterad, mottled black and mauvish white dorsad, the mid line narrowly mauvish white; spines and tubercles as in male. Metanotum and abdominal tergum I streaked and mottled with black and mauvish white, the former with a broad mauvish white median stripe. Tegmen rudiments straw mottled with dark brown. Wing rudiments straw, the raised veins flecked with black; cephalic (costal) margin mottled with black. Abdominal dorsum mainly pale grey-brown, laterocephalic flecks white, black-edged; tergum VII with black shoulder stripes, as in male but not pointed caudad, separated by narrow white median stripe; terga VIII and IX white to straw, streaked with black; tergum X straw. Ventral surface mainly olive; dark mottled areas essentially as in instar III, black and cream, connected on abdomen by some black mottling; operculum mottled black and pale olive cephalad, caudad pale olive with median carina black; sternum X pale olive. Thoracic pleura as in male. Pleural region of abdomen cream to buff, orange-brown on laterocaudal expansions of tergum VI. Legs conspicuously mottled with black, straw, and orange-brown: femora and tibiae of legs II and III, especially ventrad, with 3 black-mottled transverse zones, a distal one (the narrowest, but least mottled with straw), a central one (broader and more interrupted), and a proximal one (black largely confined to spines and carinae, the rest straw to orange-brown, the latter especially proximad); these separated by narrower straw zones having only the spines black; tarsi wholly black in distal $\frac{2}{3}$.

Isolated and crowded females in instar VII maintained essentially the pattern characters of instar VI, but in the crowded female the mauvish white areas were a purer white, the pleural region of the abdomen was mottled with buff, black, and near-white, and the orange-brown colour was a little more extensive ventrad on femora II and III.

Three males and two females reached the adult stage in the crowded culture and two males and two females in the isolated ones. Comparison of the crowded and isolated males failed to show any appreciable pattern differences. On the other hand, the crowded and isolated females did show quite marked differences, similar in character to those in instar VI but not as extreme (the female nymph showing the most

extreme high-density pattern had died in instar VII). Museum material from various localities and dates was examined which included some adults closely similar to those from the isolated cultures and a number, of both sexes, with a pattern of the high-density type much more strongly developed than in the females from the crowded culture. The most extreme of these museum adults may be assumed to approximate closely to the extreme low-density and high-density phases, which may thus be characterized (purely on the basis of dead material) as follows.

Low-density Phase (*extreme*)

Adult male.—Whole body fawn to pale olive, varying only slightly in tone from one part to another, with the exception of the following: Pronotum with a fine submarginal black line on each side. Mesonotum with dorsal spines black, also a small area mesocaudad near tegminal bases. Abdominal terga II–V dark brown with fawn laterocephalic flecks. Legs with genicular area narrowly dark brown.

Adult female.—Whole body fawn to pale olive-brown, varying slightly in tone, except as follows: Head, pronotum, and femur I brown or indefinitely marked with brown. Mesonotum with all spines and tubercles black. Tegmen and pre-anal part of wing tinged with buff. Abdominal dorsum with straw to fawn laterocephalic flecks evident only on terga III and IV. Prosternum, cervical region, and mouthparts dark brown. Meso- and metasternal spines black. Abdominal venter mainly olive-green infumate with brown. Legs faintly mottled with olive to brown, especially on tarsi, distad on tibiae, and in genicular region of legs II and III.

High-density Phase (*extreme*)

Adult male.—Antenna dark brown to black. Eye orange-brown striped with black. Head streaked with black and buff to straw; especially 2 parallel submedian black stripes, narrowly separated by straw on the occiput and diverging laterocephalad to the innermost point of the eyes. Pronotum buff to straw, heavily streaked with black. Mesonotum dorsad pale olive slightly mottled with dark olive, dark olive laterad, black mesocaudad near tegminal bases; dorsal spines black, lateral tubercles straw to orange-brown. Tegmen and pre-anal part of wing pale orange-brown. Abdominal dorsum mainly dark brown; laterocephalic flecks straw to orange, black-edged; tergum VII with broad black shoulder stripes, narrowly separated mesad; terga VIII and IX with black markings; tergum X largely black. Mesosternum mainly brown. Remainder of ventral surface straw to brown, heavily mottled with black, especially on metasternum, abdominal sternum VII, and poculum. Thoracic pleura straw mottled with black. Legs straw to pale olive, infumate and mottled with black, especially on coxae and on carinae of femora and tibiae; genicular area of femora and tibiae, especially of legs II and III, and tarsi of those legs, almost wholly black.

Adult female.—Pattern similar to male, but contrasts much sharper and black pigment much more extensive. Antenna and eye as in male. Head as in male but with more black. Pronotum cream to pale dull yellow, heavily marked with black. Mesonotum intricately mottled with black and cream to yellow, black predominating

laterad and cream to yellow dorsad; dorsal spines black; lateral tubercles cream to orange, the extreme tip sometimes black. Tegmen and wing as in male. Abdominal dorsum with the ground colour tending to pale olive-brown; laterocephalic flecks cream to white on terga II–VI, black fringes fusing mesad to form a black patch cephalad on the tergum, which makes contact with a smaller black patch mesocaudad on the preceding tergum; tergum VI mainly dirty cream to white dorsad, as though by a caudad spread of the laterocephalic flecks; tergum V with the same tendency less developed; tergum VII with broad black shoulder stripes, fused cephalad and diverging caudad, the space between black-mottled; tergum VIII heavily mottled with black, tergum IX lightly so; tergum X pale buff, lightly ticked with black. Prosternum, and mesosternum cephalad and caudad, black, finely mottled with cream to orange; remainder of mesosternum buff, the spines black. Metasternum mottled black and buff, the latter predominating caudad. Abdominal sterna and tergosternal membrane mottled black and olive; operculum pale buff, mottled with black cephalad. Thoracic pleura black, rather coarsely mottled with cream to orange and pinkish white, the black predominant on metapleuron, the other colours on mesopleuron. Pleural region of abdomen (lateral margins of terga) black, mottled with buff to orange-brown, on terga II–VI. Legs olivish straw (mainly dorsad) to pale buff (mainly ventrad), mottled with black; tarsi and genicular area of femora almost wholly black; on tibiae and ventrad on femora, the black mottling tends to resolve itself into 3 black and 2 intervening pale transverse bands, as in sixth-instar nymph.

Comparing the high-density pattern of *C. tessulata* with that of the other two species, we note the much more finely and intricately mottled character of the former, the prominence, especially in the nymph, of white or near-white markings in addition to those of the yellow-orange-buff group, and the stronger development of the high-density pattern in the adult, which in the female is as pronounced as in the nymph.

All intergrades between the low- and high-density patterns are represented in the museum material. On the whole, one gets the impression that advance along this gradient in *C. tessulata* is more a matter of an increase in the limits of the black areas wherever they occur than the blackening of different areas in succession. However, a rough sequence of changes may be recognized, especially in the very early stages of transformation from the low-density phase. The first effect seems to be a marked paling and then whitening of the regions in which later white and yellow pigment is prominent: the thoracic pleura, then the mesonotum laterad, especially on and around the lateral tubercles, and the abdominal terga VII–X. Black pigment then begins to appear on the legs, the pleura, laterad on the mesonotum, on the ventral part of the tergosternal membrane, and on the lateral margins of the abdominal terga. Late changes are an increase in the extent of black pigment on head and mesonotum (especially dorsad) and in the breadth of the shoulder stripes on abdominal tergum VII, the replacement of white by colours of the yellow-orange-buff group, especially on the thoracic pleura, and an increase in the white on abdominal tergum VI. Thus, as regards the thoracic pleura and sides of the mesonotum especially, we have quite a complicated sequence: (1) replacement of the olive-brown of the low-density phase by white, (2) invasion of the white area by black patches, (3) change of the remaining

white patches to yellow, buff, or orange-brown. It must be remembered, however, that this is not a sequence in time, but a series of end results in adults presumed to have been subjected during their development to different degrees of crowding. In the actual development of the high-density pattern in the late nymph, the first changes were apparently the yellowing of the legs and pleural region of the abdomen, the increase in the definiteness of the leg banding, and the development of the shoulder stripes on abdominal tergum VII, followed by the blackening of the mesonotum and the veins of the wing rudiments.

TABLE 5

MORPHOMETRIC DATA FOR ADULTS OF CTENOMORPHODES TESSULATA: MUSEUM MATERIAL, CLASSIFIED AS "SCARCE" OR "OUTBREAK", AND TWO FEMALES REARED IN ISOLATION FROM THE FIRST INSTAR (INCLUDED WITH "SCARCE")

F = length of femur III, C = greatest width of head. Values are the unweighted means of series means

Sex	Density	No. of Series	No. of Specimens	F (mm)	C (mm)	F/C
Male	Scarce	2	7	23·99	3·00	8·00
	Outbreak	3	8	20·57	2·89	7·13
Female	Scarce	3	5	29·52	4·73	6·25
	Outbreak	2	10	25·78	4·66	5·53

It will be clear from the above that a rating scale of the type used for *P. wilkinsoni* could readily be developed for *C. tessulata* also and used to provide an assessment of the phase level attained by any individual. The material at present available scarcely justifies this, but it may be stated that a satisfactory general correlation exists between the degree of development of the high-density pattern in the museum material and the density of the populations from which it was derived. Thus the material with low-density patterns came from the Richmond Range State Forest and Woolgoolga, N.S.W., in the early months of 1953, and from points in the Washpool State Forest in February 1955, when the insects were scarce, while that with the high-density patterns came from the Tanban and Toonumbar State Forests and Woolgoolga in the early months of 1956, when an outbreak of this species was under way in each area.

Values for F, C, and F/C were obtained for the material from each of the six sources just mentioned and for the two females reared to the adult stage in isolation. When these were classified as "sparse" or "outbreak" (the laboratory individuals being grouped with "sparse"), the results shown in Table 5 were obtained. The adult females reared in the crowded culture were too damaged for measurement.

The higher values for F C, in comparison with the other two species (Tables 3 and 4), illustrate the much more slender habitus of *Ctenomorphodes*. The sexual dimorphism is also clearly greater with respect to both F and C. The data have not been subjected to statistical analysis, but it is clear that the reduction in F and F/C as between sparse and outbreak populations is significant, while the small changes in

C are not. The direction of the change is the same as in the other species in all characters except $C\,\delta$. The sexual dimorphism ratio with respect to *C* is practically identical in the sparse and outbreak populations. Thus *C. tessulata* agrees with the other species in showing morphometric as well as pattern differences between the phases.

VI. Discussion

Kentromorphic variation has been recognized in many species of Acridoidea, a few Tettigonioidea (Chopard 1935, 1949, and others), and an increasing number of Lepidoptera (Faure 1943; Long 1953; and others), and may occur also in the Coleoptera (Utida 1956). Its demonstration in strikingly typical form in the order Phasmatodea thus adds another major group to this list; we may anticipate that the three species discussed in the present paper are by no means the only members of the group in which the phenomenon occurs.

The resemblances and differences between the three phasmatids in their kentromorphic responses, and between them and the locusts, have already been noted. The resemblances include the general character of the high-density pattern, the colours composing it, the location of the respective pigments in the different layers of the integument, and the nature of those morphometric differences that have been studied. The occurrence of white areas as a component of the high-density pattern in *C. tessulata* is paralleled in both *Schistocerca gregaria* (Forsk.) (Goodwin 1952) and *Nomadacris septemfasciata* (Serv.) (Faure 1932). Differences between the phasmatids and locusts include the rather distinct sexual dimorphism in the high-density pattern of the later instars in *Podacanthus* and *Didymuria* and the preservation of many features of that pattern in the adult stage of the same species and especially of *Ctenomorphodes*.

The investigation of phases in a new group of insects may be expected to have interesting bearings on theories regarding the mechanism and function of kentromorphic change, since it helps us to distinguish what is essential to the phenomenon from what is incidental and perhaps peculiar to a particular group. Two features of the situation in the phasmatids (typified by *P. wilkinsoni*) — the absence of overt gregariousness and the very low level of activity, even in crowds of the high-density phase — do, in fact, have such a bearing.

The first physiological theory of the pattern aspect of phase change was proposed by Uvarov (1928) as follows: Crowding of the phase *solitaria* led to increased activity and a consequent excess of waste products of metabolism. These were deposited in the cuticle in the form of the characteristic black pigment of the phase *gregaria*. As a result, the internal temperature of the insects when exposed to solar radiation was raised and their activity in consequence still further increased. The crowding also awakened the gregarious instinct and this perpetuated and increased the condition of crowding, thus putting into operation a "vicious circle" of cause and effect. As amplified by Faure (1932), this became the "locustine" or "hypermetabolic" theory. Although as recently as 1950 it still seemed probable that the hypermetabolic theory would explain the pattern differences between the phases and that most of the other differences could also be linked in one way or another with a difference in muscular

activity (Key 1950), evidence against this theory has now accumulated to the point where it is seriously threatened.

Husain and Mathur (1936*a*) had already shown that in crowded nymphs of *Schistocerca gregaria* the maximal development of black pigment did not occur at high temperatures, where, according to Uvarov, the activity level should be maximal, but at the lowest temperature employed by them, namely 24°C. Ellis (1951), while confirming experimentally for *Locusta migratoria* L. the greater activity of ph. *gregaria* than ph. *solitaria* and the greater activity of either phase when crowded than when isolated, showed that in fact activity was greatest at intermediate internal temperatures and began to decline at temperatures much below those attained by ph. *gregaria* nymphs in the sun. Moreover, extrapolation from her data indicates that little or no "marching" — the characteristic high activity of swarms of ph. *gregaria* — occurs at 24°C. Hussein (1937) found that the temperature limits for various activity levels were closely similar in *Schistocerca* and *Locusta*: in both species 24°C is near his lower threshold for "normal" activity. Chauvin (1941) and Nickerson (1956) have criticized the interpretation placed upon experiments such as those of Faure (1932) and Husain and Mathur (1936*b*), which were claimed to demonstrate phase transformation by the artificial activation of isolated nymphs. Chauvin (1941) claimed that under the conditions of his experiments the activity of crowded *Schistocerca* nymphs was little greater than that of isolated ones, although a typical ph. *gregaria* pattern developed. He suggested that the latter should be regarded as a direct "reflex response" to sensory impressions of fellow locusts. Finally, the recent work of P. and L. Joly (e.g. Joly and Joly 1954; L. Joly 1954) and Nickerson (1956) has established the existence of an endocrine mechanism as the *immediate* determinant of the phase pattern (= background—"pattern" of Nickerson) in both *Locusta* and *Schistocerca*.

Although the combined effect of this work is to make it unlikely that activity plays a necessary part in the pattern transformation, the evidence of an endocrine mechanism does not in itself exclude such participation. In fact, at least three alternative hypothetical sequences can now be constructed:

(1) external stimulation → endocrine activity → pigment change;

(2) external stimulation → muscular activity → proprioception → endocrine acitivity → pigment change;

(3) external stimulation → muscular activity → "waste" metabolites → endocrine activity → pigment change.

Quite apart from the possibility that activity may participate as indicated under (2) and (3) above, it is, of course, clear that both activity and gregariousness increase the mutual stimulation (both visual and tactile) that initiates all three sequences, and thus that both of these factors may facilitate phase transformation even if they are not essential to it.

It is in the light of these considerations that the findings on the phasmatids should be viewed. For here we have, at least in *Podacanthus*, an insect that shows kentromorphic pattern differences as marked as in any locust and yet that spends about 95 per cent. of its time inactive, even in crowds of the high-density phase, and

is not overtly gregarious.* It is scarcely conceivable that the difference between 2·8 and 5·5 per cent. in the amounts of activity of isolated and crowded nymphs (even if this were significant—see Section III(*a*)) could give rise to an adequate stimulus, either proprioceptive or through "waste" products of energy metabolism, to activate a presumed endocrine mechanism—especially when one considers the probable range of activity levels occurring among isolated nymphs subjected to various environmental conditions. Similarly, one cannot suppose that either increased activity or the statistical trend towards a non-random distribution can play any important part in increasing the level of mutual stimulation resulting from an increase in population density. The nature of this stimulation has not been investigated in the phasmatids. It is unlikely to be olfactory, since the individual wire-gauze cages were placed only a few inches apart, yet none of the isolated nymphs showed any trace of the high-density pattern. Probably it consists of tactile stimuli resulting from the exploratory wavings of the fore legs of adjacent nymphs and other forms of mutual contact, possibly reinforced by visual stimuli. These stimuli must be supposed to reach an intensity adequate for the production of the extreme high-density phase at an average density of only one nymph per 15-in. branchlet and for partial phase transformation at much lower densities (Section III(*c*) and Fig. 1).

Thus in the Phasmatidae we have a kentromorphic mechanism, typical as to its pattern end-products, in which there is probably a direct link, through the central nervous system, between sensory stimulation and an endocrine organ (sequence (1) above), and in which neither gregariousness nor increased muscular activity plays a significant part. This suggests very strongly that sequence (1) will be found to represent the situation in locusts also, and that there, too, activity and gregariousness, while in that case playing an important facilitating role, are not essential to the process of pattern change.

With regard to the morphometric characters, Faure (1932) and Uvarov and Thomas (1942) have espoused the theory that the constant pull of the muscles upon the growing exoskeleton in locust nymphs exhibiting a high degree of activity could account for the deformations expressed by the various indices. Joly and Joly (1954), on the other hand, would explain the differences on an endocrine basis. They hold (see also Kennedy 1956) that the ph. *solitaria* adult of *Locusta* is morphometrically neotenic in comparison with the ph. *gregaria*, being held in that condition by the activity of the corpora allata: the latter is inhibited in ph. *gregaria* by nervous impulses resulting from mutual stimulation. It should be pointed out that both P. Joly (1956) and Kennedy (1956) ignore the fact that with respect to the ratio *F*/*C*, now known to be the best phase character in this species, it is the adult ph. *gregaria*, not *solitaria*, that is, on the Jolys' own data (see P. Joly 1956), nearer to the nymphal condition.

The contribution of the Phasmatidae in this field is to indicate that here we have, in three species, changes in *F*/*C* quite analogous to those in locusts, but with no appreciable difference in activity.

* By "overt" gregariousness is meant a condition that would be immediately recognized as such from the behaviour of the insects, not one that can only be inferred from a statistical study of distributions. The latter is probably very widespread among animals.

On the question of the biological function of kentromorphic pattern change, two theories have been proposed by workers on locusts. The first is linked with the hypermetabolic physiological theory (Uvarov 1928) and claims that the ph. *gregaria* pattern, by raising the body temperature, increases activity and gregariousness in a vicious circle, thus promoting and maintaining the characteristic migratory behaviour of swarms during an outbreak. The second has been advanced quite recently by Kennedy (1956), who draws attention to the great conspicuousness of the *gregaria* pattern and suggests that it may have the function of facilitating the discriminative refinement of the visual compensation reaction (Kennedy 1939), thus again intensifying gregariousness and activity and promoting migration. Both these theories assume that gregariousness, high activity, and extended migrations have a positive selection value, i.e. are adaptive, when locust populations become large. In general this assumption is no doubt justified. It has been argued by Key (1945), Ellis (1951, 1953), and Kennedy (1956), among others. It holds primarily for environments where food is abundant in restricted localities separated by extensive, practically foodless tracts — i.e. primarily for environments that are at least seasonally arid or semi-arid. Such environments have produced, in every continent, species of Acridoidea, often taxonomically unrelated, with the characteristic ecological and behavioural attributes of a locust. Indeed, the locust biotype is as characteristic, and presumably as adaptive, as that of the gregarious grazing mammal. Granted, then, that gregariousness and activity are adaptive in the locust's environment, we must reject the first theory for the reasons already discussed, but Kennedy's theory deserves further attention.

There is no doubt about the conspicuousness of the high-density nymphal pattern in both locusts and phasmatids and to a less degree, perhaps, in other groups. We are not dealing with a uniform or random distribution of pigment deposited, as a matter of physiological convenience, in the integument, but with a definite, strongly contrasted, constant pattern of bright colours, comprising typically black and a colour of the yellow-orange-red-brown group. A complex physiological mechanism has been evolved with the specific function, as it would seem, of bringing this pattern into being in dense populations and dissipating it in sparse ones. There is even evidence that in different species essentially the same pattern effect may be produced by different mechanisms. Thus the yellow areas of the *gregaria* pattern of *Schistocerca gregaria* are due to carotenoids, while the corresponding orange-brown areas of *Locusta migratoria* are believed to be due either to a tanning of the cuticular protein by orthoquinones, or to a weak scattered melanization, but certainly not to carotenoids (Goodwin 1952). Convergence of this kind provides evidence of the prime importance of the common end-result of the converging processes. It would be of great interest to know the pigments responsible for the sharply contrasted yellow and bright orange-red colours of the *gregaria* pattern in nymphs of *Nomadacris septemfasciata* (see Faure 1932).

A further reason for regarding conspicuousness as, so to speak, the specific aim of the high-density pattern is afforded by the fact that the insects in which kentromorphic phases occur belong to strongly procryptic groups and are themselves procryptically coloured in the low-density phase. Among the Acridoidea, efficient

mechanisms for the active accommodation of the body colour to that of the background have been evolved (cf. Fraser 1932; Ergene 1950; Burtt 1951), while the Phasmatodea are notorious for their extreme procryptic adaptations in both colour and form. That these adaptations do in fact have considerable survival value has been convincingly demonstrated by Isely (1938) and Ergene (1953) for grasshoppers and the whole subject has been comprehensively reviewed by Cott (1940). Thus the substitution of a highly conspicuous pattern for this elaborate adaptation for concealment cannot possibly be regarded as a matter of chance, or as adaptively neutral but must possess a marked survival value in the special conditions under which it arises.

All these considerations would be consistent with Kennedy's theory. However, as soon as we bring the behaviour of the Phasmatidae into the picture and follow Kennedy to the next step, the theory immediately breaks down. For the three phasmatids studied are not active or gregarious, there is no migration, and the females of *Didymuria* and *Ctenomorphodes* are flightless. Very high population densities lead to complete defoliation of the forest over large areas and the phasmatids survive only around their fringes. If Kennedy's theory cannot be applied at all to the Phasmatidae, in which the high-density pattern is so characteristically developed, then it is most unlikely that it affords the basic explanation of the phenomenon in locusts either.

At this point we may consider a possibility that has not hitherto been canvassed. Can it be that the conspicuous high-density pattern has an aposematic function? The banding and mottling with black and yellow to red that characterizes this pattern in its typical development is, in fact, the classical pattern of aposematic animals of all groups.

Such an explanation would be consistent with a number of facts. In the first place, procryptic adaptations are effective only when their bearers are not too abundant, when they confine themselves to the narrow habitat to which they are adapted, and when they spend much of their time at rest (Cott 1940). These conditions are fulfilled by sparse populations of the low-density phase of locusts and phasmatids. As soon as such insects become massed together, as the phasmatids do purely from an increase in numbers and the habit of sitting on the outer foliage, and still more, as the locusts do because of their gregariousness, the effectiveness of the adaptation becomes much reduced. When, further, locust swarms leave the habitat of the ph. *solitaria* and march actively over open ground, the effectiveness is completely lost. On the other hand, aposematic animals are well known for their habit of congregating together in conspicuous masses, presumably because in this way the "warning" colouring has greater impact upon a potential predator. Species of aposematic Acrididae in the genera *Zonocerus* Stål, *Zonocopris* Stål, *Romalea* Serv., and others behave in this way in the nymphal stage (Jones 1934; Cott 1940), and Wegner (1955) has recorded similar behaviour in aposematic species of the phasmatid genus *Myronides* Karp. Finally, kentromorphic variation is characteristic precisely of species that sometimes occur at very high levels of abundance (and are then often gregarious), although at other times they may be rare. Thus the theory would imply that the colour-pattern aspect of kentromorphic variation had evolved as a mechanism for effecting a switch-over from procryptic to aposematic adaptations in nice correlation with the population densities under which each of these adaptations has survival value. It would be a

theory of facultative aposematism. We have already noted that in *Podacanthus* the phase transformation takes place within quite a narrow density range; in locusts the density is rapidly increased by the onset of gregarious aggregation, which triggers a relatively abrupt pattern change. Thus the potentially awkward intermediate phase (from the point of view of the theory) tends to be quite transient.

Direct evidence in favour of this theory is practically nil. We know that procryptic species of Acridoidea and Phasmatodea are readily eaten by many birds and some mammals, while aposematic species are rejected (Carpenter 1921; Isely 1938; etc.). We do not know whether acceptability is reduced in the high-density phase, although it may well be that bird predation on locust swarms is not as high (apart from a few specialist locust feeders) as one might expect. Protection might be expected merely from the fact that the pattern is of the aposematic type, without any parallel reduction in actual palatability (see Carrick 1936; Cott 1940), although such a reduction is not to be excluded. However, it is generally regarded as a condition for effectiveness in this "Batesian mimicry" that the mimic should be less abundant than the models, and this could not be true of locusts or phasmatids, at least at the times when the high-density phase is in evidence.

A situation that may be in some respects parallel to that of kentromorphic pattern change has been discussed by Poulton (1908). In butterflies of the genus *Precis* Hübn., a procryptic "seasonal" phase produced in the dry season alternates with a conspicuous and probably aposematic wet-season phase. In the wet season the butterflies are more numerous, as are most other insects, and each individual is subjected to a lower predation pressure than in the dry season, when insects are scarce. Poulton assumes that the species of *Precis* are *relatively* distasteful — sufficiently so to render an aposematic pattern partially protective in the wet season, although it fails to protect, and is replaced by procryptic adaptations, in the dry-season phase. That neither Acrididae nor Phasmatidae are unreservedly acceptable to vertebrate predators is suggested by their habit of regurgitating a large drop of their crop contents when seized and then proceeding to wipe this on to the fingers with every sign of deliberation. The smallest trace of this material, if subsequently rubbed into the eye, results in considerable irritation. The spines on the hind tibiae of Acrididae may also be a partial deterrent.

Techniques for demonstrating selective feeding by birds have been developed by Isely (1938), Jones (1934), Carrick (1936), and others. It should therefore not be too difficult to test the aposematic theory. Phasmatidae may well prove to be well adapted to such tests.

Phase rating scales, such as the one developed here for *Podacanthus wilkinsoni*, may have a practical application in control work on injurious phasmatids, since they should enable the economic entomologist to assess the density of an infestation from a suitable sample of nymphs or adults collected by untrained personnel.

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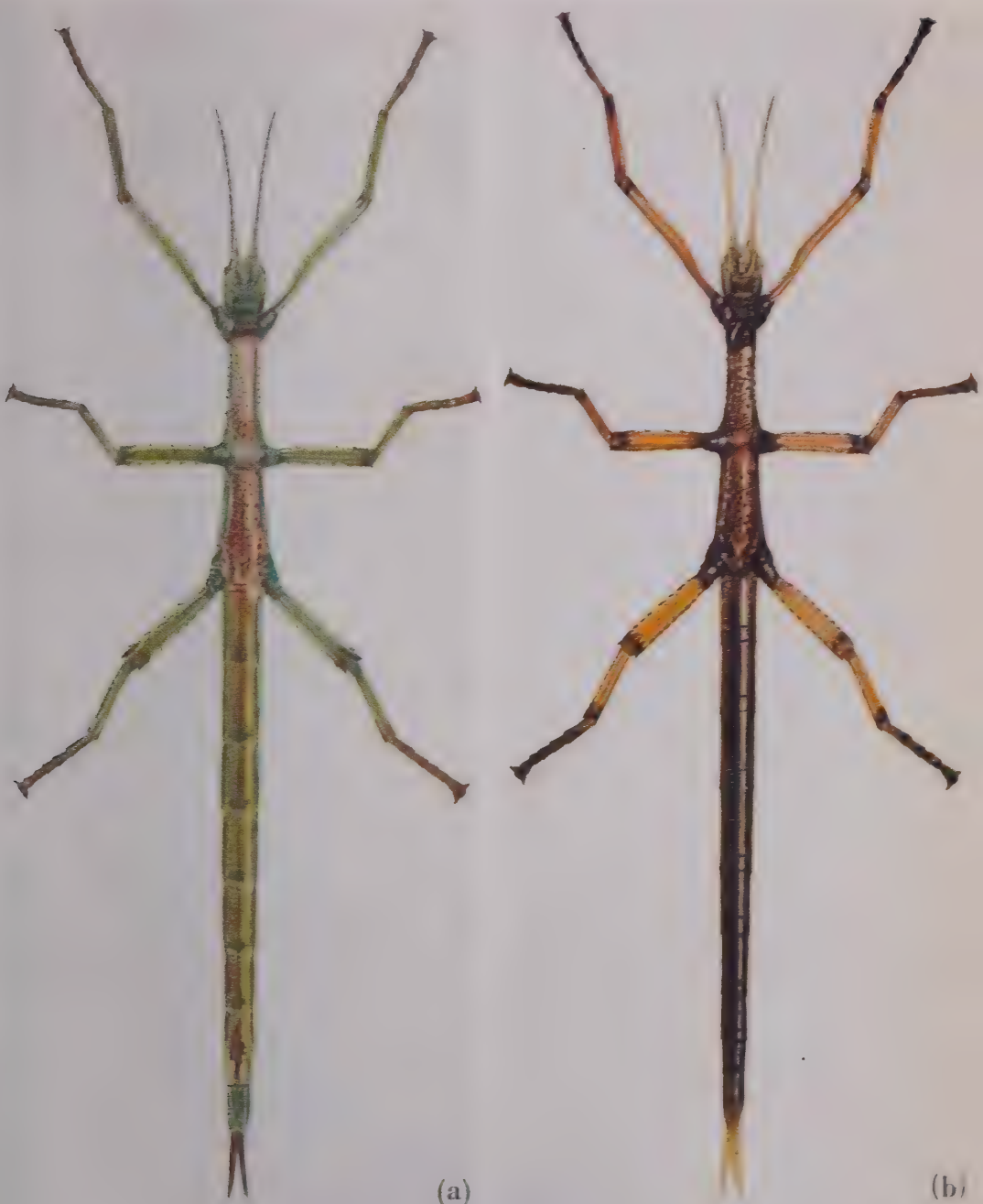
KENTROMORPHIC PHASES IN PHASMATODEA

Podacanthus wilkinsoni. Male nymphs in instar A−2, dorsal view. (*a*) Low-density phase, reared in isolation; (*b*) high-density phase, reared in a crowd. × *c*. 3.

KENTROMORPHIC PHASES IN PHASMATODEA



Podacanthus wilkinsoni. Male nymphs in instar A—2, ventral view. (*a*) Low-density phase, reared [illegible]. (*b*) high-density phase, reared in a crowd. [illegible].

KENTROMORPHIC PHASES IN PHASMATODEA

Didymuria violescens. Male nymph in final instar, high-density phase. (*a*) Dorsal view; (*b*) ventral view. c. 1·3.

CYTOGENETICS OF THE GRASSHOPPER *MORABA SCURRA*

I. MEIOSIS OF INTERRACIAL AND INTERPOPULATION HYBRIDS

By M. J. D. White*

[*Manuscript received April 24, 1957*]

Summary

Numerous F_1 hybrids between the races of *Moraba scurra* Rehn with $2n♂ = 15$ and $2n♂ = 17$ were reared in the laboratory and in artificial colonies established in nature. Most F_1 males have almost completely regular meiosis, their chiasma frequency being essentially normal. These individuals show a trivalent, composed of an "*A*" and a "*B*" chromosome of the 17-chromosome race paired with the corresponding limbs of an "*AB*" chromosome derived from the 15-chromosome race. Disjunction of the trivalent is usually extremely regular. Occasionally either the *A* or the *B* chromosome fails to pair with the *AB*.

In hybrid individuals which happen to have their *CD* chromosome pair heterozygous for the Blundell rearrangement (i.e. "standard/Blundell") the proximal end of the *A* chromosome is occasionally paired with the Blundell-carrying *CD* chromosome. This suggests that the evolutionary "dissociation" of the *AB* into separate *A* and *B* chromosomes, which is present in the 17-chromosome race, was not a simple fragmentation but a translocation involving a Blundell chromosome, and that the *A* chromosome received its centromere from the Blundell element.

Various types of abnormal synapsis occur in hybrids between individuals from populations situated several hundred miles apart, even when both belong to the 15-chromosome race.

Chiasma-formation in *CD* bivalents homozygous and heterozygous for the three known sequences of the *CD* chromosome (standard, Blundell, Molonglo) suggests that Blundell and Molonglo are both related to standard as pericentric inversions.

The bearing of these data on the history of the species is discussed.

I. Introduction

The adaptive chromosomal polymorphism of the Australian grasshopper *Moraba scurra* Rehn (Orthoptera: Eumastacidae) was described in a recent paper (White 1956) and certain aspects of it will be considered in greater detail in the second paper of this series (White 1957*b*). There are two races of this species which differ in chromosome number, the eastern one (which inhabits the Southern Tablelands of New South Wales and a portion of northern Victoria) having $2n♂ = 15$, while the western race (which occupies a part of the south-western slopes of New South Wales) has $2n♂ = 17$. The dividing line between the two (shown in White 1956, Fig. 1; White 1957*b*, Figs. 1, 2; White and Chinnick 1957, Fig. 1) runs southwards from near Cowra, N.S.W., to the Yass area and then south-west to the vicinity of Holbrook, N.S.W., but collecting has not been extensive enough to determine its exact course throughout the whole length of about 150 miles. Cytologically, the eastern race has a pair of large metacentric "*AB*" chromosomes, which is represented by two pairs of acrocentric elements, "*A*"

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and "*B*" in the western race, thus accounting for the difference in chromosome number.

No mixed colonies, in the strict sense, i.e. ones with a significant number of both kinds of individuals and the heterozygotes between them, have been found in nature, although a single 16-chromosome male heterozygous for a "broken" *v.* "fused" *AB* was found in 1956 in a colony of the eastern race situated about 900 yards away from one of the western race (White and Chinnick 1957). Apart from this heterozygote, occurring almost on the dividing line between the two races, six others of similar constitution have been found within the territory of the eastern race, but far removed from that of the western race. A seventh individual in which the *AB* was likewise broken, having participated in a translocation with one of the smaller elements, was found at Hall, A.C.T., which is also geographically distant from the interracial boundary (White 1956). These six (or seven if we include the individual from Hall) occurred in a total of 6178 individuals of the eastern race which have now been examined cytologically. No heterozygotes for broken/fused *AB* have been encountered in 1851 males of the western race.

Whereas the heterozygote found in close proximity to the interracial boundary almost certainly arose through interracial hybridization in nature, the six others are not so easily explained. They were found in four different colonies (Paddy's River "B", Komungla "B", Royalla "A", and Michelago) and it was formerly considered uncertain whether they were remnants, as it were, of a former condition in which some populations showed a higher degree of polymorphism for a "fused" *v.* a "broken" *AB* (White 1956). In this case the "breakage" of the *AB* might have occurred once only in the history of the species (or twice, if we include the Hall male). Alternatively, it seemed possible that several independent dissociations of the two limbs of the *AB* might have occurred and, even, that fragmentation of the *AB* in its centromere region might be a relatively frequent occurrence.

In order to obtain more fused broken *AB* individuals a number of pair-matings between members of the eastern and western races were set up in the laboratory. In addition, several artificial colonies were established at suitable localities in nature, in some instances by "contaminating" an existing population with males obtained from a different locality, in other cases by taking males from one locality and female nymphs (i.e. virgins) from another and releasing them at a third locality where the species was not already present.

It was hoped from these hybridization experiments (1) to obtain evidence as to the exact nature of the "dissociation" of the *AB* which seems to have occurred in the western race (or, alternatively, evidence that a "fusion" occurred in the eastern race), and (2) to find out whether the meiotic behaviour of artificially produced broken fused *AB* heterozygotes would be similar to that of the few heterozygotes found in nature.

In addition to the interracial matings, a number of intraracial crosses between individuals of the 15-chromosome race from widely different localities were also made. Since *M. scurra* is an apterous insect with very feeble powers of locomotion and specialized ecological requirements which restrict it to habitats of a particular type, it was felt that a certain amount of cryptic cytological differentiation might

have taken place in the course of its undoubtedly long evolutionary history as a species. Such cryptic differentiation might have been expected to include various types of structural rearrangements (e.g. inversions and translocations) for which the populations of particular regions would be homozygous.

The chromosomal rearrangements which occur in the "*CD*" and "*EF*" chromosomes of both races have already been described and figured (White 1956). There are two alternative types of *EF* chromosome, a metacentric type which we call Standard (*St*) and an acrocentric which we call Tidbinbilla (*Tid*) (it having been first collected near a mountain of that name). Three sequences are known for the *CD* chromosome, which have been named Standard, Blundell (*Bl*), and Molonglo (*Mol*). At the time when the hybridization experiments were carried out, the Molonglo sequence was only known with certainty from one locality (near Bungendore, N.S.W.) from which the Standard *CD* was absent. Thus one of the hybridization experiments consisted in introducing some males carrying Standard *CD* chromosomes into the Bungendore colony, with the objective of recovering some *St*/*Mol* heterozygotes in the F_1. Subsequently, four additional colonies have been found in which Molonglo occurs, and in two of these the Standard *CD* is also present. It has thus been possible to study both "synthetic" and "natural" *St Mol* heterozygotes and to obtain some information bearing on the problem of the relationship of the three sequences.

II. Material and Methods

M. scurra can be kept in the laboratory in jars with gauze or muslin covers, using *Helichrysum apiculatum* D. Don as food. Under such circumstances the mortality of nymphs and adults is very low (until the senile stage is reached, after about 9 months). This low mortality is probably associated with the fact that the insect is tolerant of a wide range of humidities. On the other hand, the eggs obviously require very careful management, and we did not succeed in discovering satisfactory techniques for ensuring a high percentage hatch (in all our laboratory crosses there was a large egg mortality).

The laboratory-reared hybrids which were studied cytologically are shown in Table 1. Matings 1–5 were between members of the eastern and western races, i.e. all the offspring of these crosses are necessarily heterozygous for the broken-fused *AB* condition. These will be referred to as interracial crosses. Matings 6 and 7 were between members of the same (eastern) race and will be called interpopulation crosses. A map showing the localities where the parental material was collected has already been published (White 1956, Fig. 1). In addition to these laboratory-bred hybrids, four hybridization experiments were carried out under field conditions: three of these involved interracial crosses and one an interpopulation cross (see Section III for details).

The testes of the laboratory-reared hybrids were fixed in Navashin's fluid, sectioned at 16 μ, and stained with crystal violet by Newton's method. Those of the hybrids from the field experiments were made into aceto-orcein squash preparations and mounted in "Euparal" after freezing with solid carbon dioxide by the method of Conger and Fairchild (1953).

III. The Field Experiments

(*a*) *The Windellama Experiment*

The population of *M. scurra* in the small cemetery at Windellama, N.S.W., has been studied for several years. It is a very small colony of the eastern race,

Table 1

CYTOLOGICAL DATA ON LABORATORY-BRED HYBRIDS

Pair-mating	Geographic Origin of:		Individual No.	Cytological Constitution of Chromosomes:			Chiasma Frequency per Cell*
	Mother	Father		*AB*	*CD*	*EF*	
1	Tangmangaroo	Royalla "A"	1803	Broken/fused	*St*/*Bl*	*St*/*St*	8·59±0·05
2	Wallendbeen	Murrumbateman	1804	,,	*St*/*St*	*St*/*St*	9·54±0·06
	,,	,,	1818	,,	*St*/*St*	*St*/*St*	10·19±0·06
3	Boorowa "A"	Murrumbateman	1807	,,	*St*/*St*	*St*/*St*	9·63±0·06
	,,	,,	1808	,,	*St*/*Bl*	*St*/*St*	8·75±0·05
4	Kyeamba	Collector	1824	,,	*St*/*Bl*	*St*/*St*	8·47±0·07
	,,	,,	1826	,,	*St*/*Bl*	*St*/*Tid*	7·70±0·07
5	Bowning "B"	Murrumbateman	1823	,,	*St*/*Bl*	*St*/*St*	9·01±0·04
6	Yass	Murrumbateman	1809	Fused/fused	*St*/*St*	*St*/*St*	9·98±0·001
7	Wodonga	Hall	1806	,,	*St*/*Bl*	*St*/*St*	8·51±0·05
	,,	,,	1825	,,	*St*/*Bl*	*St*/*St*	7·98±0·07
	,,	,,	1827	,,	*St*/*Bl*	*St*/*St*	7·98±0·10

Comparison of Chiasma Frequencies of Hybrids with Mean Chiasma Frequencies of Non-hybrids

Genotype	15-chromosome Race		17-chromosome Race	
	Mean	S.E. of the Mean of Means	Mean	S.E. of the Mean of Means
St/*St*, *St*/*St*	9·496†	±0·078	9·971‡	±0·408
St/*Bl*, *St*/*St*	8·584†	±0·016	9·488§	±0·157
Bl/*Bl*, *St*/*St*	9·000†	±0·011	9·308‖	±0·200

* Based on 100 first metaphases in the case of each individual, except No. 1826 (of which only 50 first metaphases were analysed) and No. 1827 (of which only 68 were analysed).

† Based on 10 first metaphases from each of 25 individuals.

‡ Based on 10 first metaphases from each of 7 individuals.

§ Based on 10 first metaphases from each of 8 individuals.

‖ Based on 10 first metaphases from each of 12 individuals.

consisting of only a few dozen individuals in 1954, 1955, and 1956. In 1955, 24 males were removed (at a time when the females were still nymphs) and replaced by 30

males of the western race from Murringo, N.S.W. On May 4, 1956, the locality was visited in order to collect a sample of males. It was found that the area where most of the Murringo males had been released had been ploughed up, leading to changes in the vegetation. Eighteen males were collected in another part of the cemetery, but none were heterozygous for broken/fused *AB*. This was the only one of the field experiments which was apparently unsuccessful. It is recorded here in case any descendants of the Murringo males are found in future years.

(*b*) *The Lake George Experiment* I

Eighty-five females of the western race from Murringo cemetery, together with 70 males of the eastern race from Paddy's River "B", were liberated at a locality near the north end of Lake George, N.S.W., in June 1955 (at this date all the individuals would have been virgins). The locality, although apparently suitable for the species, did not have an indigenous population of *M. scurra*, and none appears to exist in the vicinity, so it was considered certain that contamination would not occur during the next few years (contamination from the next experiment is possible but unlikely for several years and then only if one or other population builds up to large numbers).

All the F_1 individuals would necessarily be heterozygous for the fused/broken *AB* condition. On the basis of the chromosomal frequencies in the populations from which the parents were drawn, it was estimated that 40·7 per cent. would have the *CD* pair *St*/*St*, 52·8 per cent. would have it *St*/*Bl*, and 6·5 per cent. would have it *Bl*/*Bl*. And, on the same basis, 79 per cent. were expected to have their *EF* pair *St*/*St* and 21 per cent. to have it *St*/*Tid*. Large sampling errors are, of course, involved in making these estimates.

On May 9, 1956, a thriving colony of F_1 individuals was found. A sample of 10 males contained 7 *St*/*Bl*, 2 *St*/*St*, and 1 *Bl*/*Bl* for the *CD* chromosome. Nine had the *EF* chromosome pair *St*/*St* and only one had it *St*/*Tid*. The experiment proves that hybridization between the two races will occur freely when they are brought in contact and that the fertility of such matings is high under natural conditions. The composition of the F_1 population as far as the rearrangements in the *CD* and *EF* chromosomes are concerned does not depart significantly from expectation on the basis of the small sample examined.

(*c*) *The Lake George Experiment* II

Thirty-one females from Young cemetery (western race) and 31 males from Paddy's River "B" (eastern race) were liberated in June 1955 (when they were all virgins) at a point about 200 yd from the site of the previous experiment and separated from it by a strip of land which was unsuitable for the species and included a small creek and a gravel road. As in the previous experiment there was no indigenous population. Contamination from the previous experiment, although theoretically possible, is unlikely to occur for several years and then only if one or the other population builds up to large numbers.

The locality was visited again on May 9, 1956. Only about 10 F_1 individuals were seen, but more were probably concealed in the rather dense vegetation. However, this

colony was certainly smaller than that in the previous population, and its ultimate survival more precarious. Four males were collected for cytological examination (Tables 2 and 3).

(*d*) *The Bungendore Experiment*

Six miles south of Bungendore, N.S.W., there is a colony of *M. scurra* whose *CD* chromosomes, in 1955, were 74·25 per cent. Blundell and 25·75 per cent. Molonglo (White 1956). A total of 200 males were removed from the population up to April 26, 1955. On that date, 250 males from Paddy's River "B" were released by scattering them throughout the area occupied by the Bungendore population. The object of this was to introduce some Standard *CD* chromosomes and so to create a population containing all three types of *CD*. Six days later 54 males were captured. Of these 51 were analyzed cytologically. Nine carried the Molonglo rearrangement (which does not occur at Paddy's River) and hence belonged to the indigenous population. Fourteen carried the Standard *CD* sequence and were consequently derived from the introduced Paddy's River males. The remaining 28 had their *CD* chromosome pair homozygous for the Blundell sequence and might hence have been either "natives" or "aliens". There are various ways of estimating how many of these 28 belonged to these two categories, but they agree in assigning rather more than half of them to the natives (10 of them were heterozygous for the Tidbinbilla rearrangement and most of these, at any rate, must have been aliens). Thus we may roughly estimate that half of the 51 individuals were natives and half aliens.

This result suggests that the males in the colony numbered about 500 before the 54 were removed from it and that the total population was probably about 1000 (assuming equal numbers of males and females).

It was consequently expected that about half the individuals present in 1956 would be F_1 interpopulation hybrids. Not all of these would be cytologically recognizable as such, but about 23 per cent. were expected to carry a Standard *CD* chromosome. In point of fact, only three out of 31 examined did so. Thus our previous estimate of the original population may have been too low and a figure of 1500 now seems plausible.

IV. Meiosis of the Male Hybrids

The spermatogenesis of all the interracial and interpopulation hybrids is histologically normal. The testes are no smaller than usual, the course of the meiotic divisions is regular, and the only abnormalities (and these are rare) are at the cytological level.

In the interracial hybrids we expect to find at meiosis a trivalent composed of the *A* and *B* elements associated with the corresponding limbs of the *AB* chromosome (Fig. 1(*a*)). This expectation is, in fact, realized in the great majority of the cells (Tables 1 and 2). In such trivalents the *AB* apparently forms a distal chiasma in each arm, one with the *A* chromosome and one with the *B*. By first metaphase both of these may be completely terminalized, but in many cells one is still interstitial. If one examines prometaphases of the first meiotic division, one might get the impression that a considerable proportion of the trivalents were wrongly orientated (i.e. with the *A* directed towards one pole and the *B* towards the other). Such mal-

orientation seems, in most instances, to be only a temporary feature and by mid or late metaphase it has usually been replaced by the "correct" orientation, with the *A* and *B* directed towards the same pole and the *AB* towards the opposite one.

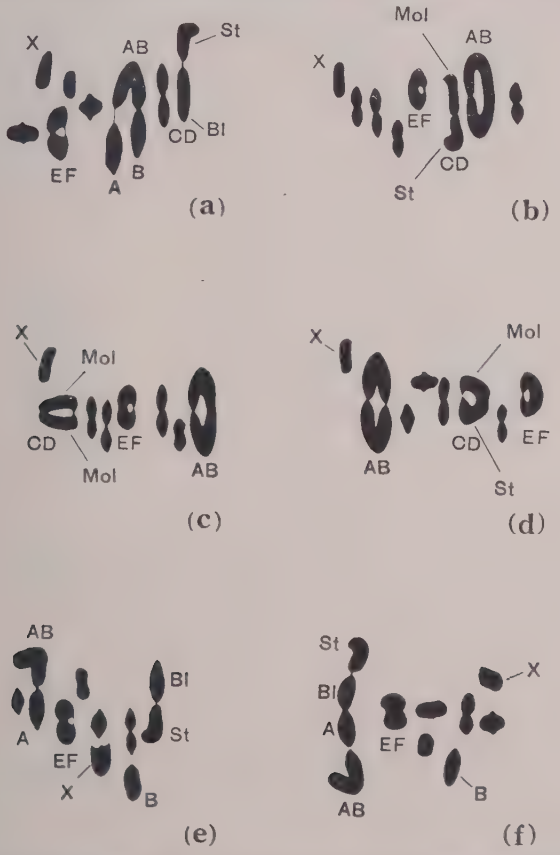

Fig. 1.—First metaphases (in side view) of various individuals of *M. scurra*. (*a*) From individual No. 1803, showing the trivalent; (*b*) from a Taemas Bridge individual with the *CD* bivalent heterozygous for *St*/*Bl* and showing only a distal chiasma; (*c*) from a Bungendore individual with the *CD* bivalent homozygous for Molonglo and showing both proximal and distal chiasmata; (*d*) from a Bungendore × Paddy's River "B" hybrid, heterozygous for *St Mol* and showing both proximal and distal chiasmata in the *CD* bivalent; (*e*) and (*f*) from individual No. 1824, with the *B* chromosome present as a univalent (in (*f*) there is a chain of four chromosomes).

Exceptions to the presence of a trivalent are of two kinds. In 271 cells, out of 4723 first metaphases of interracial hybrids examined, one of the acrocentric chromosomes (either *A* or *B*) was unpaired and present as a univalent at first metaphase, while in five cells *A*, *AB*, and *B* were all univalents (Tables 2 and 3). However, 217 of the 271 cells with a univalent were in a single individual, the offspring of a Kyeamba mother and a father from Collector, N.S.W. Excluding this individual, the cells with a univalent only amount to 1 per cent. of the total, instead of 6 per cent.

TABLE 2

FREQUENCY AND BEHAVIOUR OF VARIOUS TYPES OF CHROMOSOMAL CONFIGURATIONS IN "SYNTHETIC" AND NATURALLY OCCURRING *AB* HETEROZYGOTES WITH THE *CD* PAIR *St/St* OR *Bl/Bl*

All except Nos. 1831 and 1832 "synthetic" heterozygotes (see Table 1 for parentage). No. 1831, a natural heterozygote from Royalla; No. 1832, one from Michelago

Individual No.	*A–AB–B* Trivalent	Bivalent (*A–AB* or *AB–B*) and Univalent	Other Configurations
1804*	549	3	—
1818*	465	—	—
1807	306	—	—
III–2†	242	—	—
III–9*	111	2	—
IV–2†	150	2	—
IV–4†	83	—	—
1831†	174	—	—
1832*	59	2	1‡
Total	2139	9	1

* *CD* pair *St/St*. † *CD* pair *Bl/Bl*. ‡ *A*, *AB*, and *B* all univalents.

TABLE 3

FREQUENCY AND BEHAVIOUR OF VARIOUS TYPES OF CHROMOSOMAL CONFIGURATIONS IN "SYNTHETIC" *AB/A,B* HYBRIDS WITH THE *CD* PAIR *St/Bl*

Individual No.*	First Metaphases with:				
	A–AB–B Trivalent	Bivalent (*A–AB* or *AB–B*) and Univalent	Quinquevalent (*St–Bl–A–AB–B*)	Quadrivalent (*St–Bl–A–AB*) and Univalent *B*	Three Univalents (*A*, *AB*, and *B*)
1803	403	4	5	—	—
1808	418	4	13	—	—
1823	251	1	25	—	—
1824	273	217	2	4	4
1826	81	11	2	—	—
III–1	53	4	—	—	—
III–3	62	4	—	—	—
III–5	184†	3	2	—	—
III–7	128	—	—	—	—
III–8	34	—	—	—	—
III–10	314‡	1	1	—	1
IV–1	127	15	—	—	—
IV–3	159	—	5	—	—
Total	2487	264	55	4	5

* See Table 1 for the parentage of these hybrids.

† *CD* pair asynaptic in two of these cells.

‡ *CD* pair asynaptic in one cell.

A second type of exception to the presence of an *A–AB–B* trivalent occurs only in those hybrids that happen to have the *CD* chromosome heterozygous (i.e. Standard/Blundell). In about 2 per cent. of the first metaphases of such individuals a quinquevalent (chain of five) is seen (Table 3, Fig. 2). Such quinquevalents have been found in eight different hybrid individuals, whose parents came from a number of different localities. The sequence of the five chromosomes in the chain is presumably always the same; but since we cannot distinguish the *A* from the *B* element by any difference of length or shape, we shall say that the sequence is Standard *CD*—Blundell *CD–A–AB–B* (i.e. we shall define *A* as the chromosome which pairs with the Blundell element).

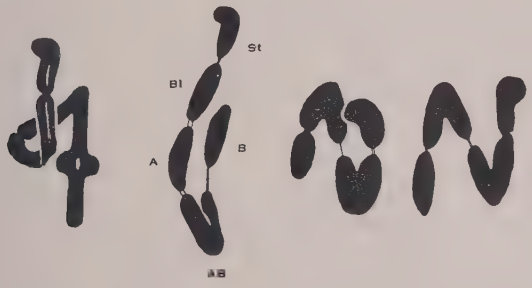

Fig. 2.—Quinquevalents from interracial hybrids Nos. 1808, 1823, and 1824, showing characteristic orientations at first metaphase.

The "anomalous" chiasma between the *A* and the Blundell chromosome is formed between the proximal ends of both elements. It has not been possible to obtain critical evidence as to whether this chiasma is between the short arms or on the other side of the centromeres, in the main arms.

In the Kyeamba × Collector hybrid (in which almost half the first metaphases show a univalent *A* or *B* and the corresponding *AB–B* or *A–AB* bivalent) four cells had a quadrivalent (*St–Bl–A–AB*) and a univalent *B*. Although carefully sought for, no cells with a *St–Bl–A* trivalent and an *AB–B* bivalent were found. Thus we conclude that in this individual the lack of pairing is between the *B* limbs rather than between the *A*'s and that the majority of univalents are consequently *B*'s.

The chain of five may orientate itself in a zig-zag on the spindle (Fig. 2). But being very long, it rather frequently orientates itself in other ways, some of which will certainly give rise to non-disjunction at anaphase.

There is a good deal of heterogeneity between individuals in regard to the number of cytological abnormalities seen at first metaphase. Thus individual No. 1823 showed 10 per cent. of cells with quinquevalents, while several showed none at all. Whether the large number of univalents present in the Kyeamba × Collector hybrid No. 1824 indicates the presence of cryptic cytological differences between the parents (possibly "invisible" paracentric inversions) or genic interference with synapsis or chiasma formation cannot be determined.

The extent to which fertility would be reduced in the interracial hybrids by the cytological anomalies seen can be estimated only roughly. Omitting the anomalous Kyeamba × Collector individual, we might estimate that failure of pairing between *A* or *B* and the *AB* occurs in 1 per cent. of spermatocytes, while quinquevalents occur in 2 per cent. of the cells of *St*/*Bl* individuals but not at all in homozygotes for either Standard *CD* or Blundell *CD*. We may guess that approximately half of the first anaphases with either of these anomalies are non-disjunctional.

It is rather difficult to estimate what percentage of trivalents become irrevocably mal-orientated at first metaphase, since, as we have already pointed out, a considerable number go through a temporary phase of mal-orientation. However, in hybrid No. 1818 (see Table 1) the *A–AB–B* trivalent rather frequently has both proximal and distal chiasmata (i.e. it shows three or even four chiasmata). In such cases mal-orientation may be present right through the first metaphase stage and presumably leads to non-disjunction. Individual No. 1826 also showed about 16 per cent. mal-orientation of the trivalent in what appeared to be late first metaphases.

We may accordingly expect that some *St*/*St* and *Bl*/*Bl* interracial hybrids will have a fertility about 99 per cent. of normal, but that in others mal-orientation of trivalents will reduce it to perhaps 90 per cent. In *St*/*Bl* heterozygotes the formation of quinquevalents will still further reduce fertility. Except for the Kyeamba × Collector hybrids (whose fertility may have been about 70–80 per cent. of normal), all the interracial hybrids examined probably produced over 90 per cent. euploid sperms.

The "interpopulation" hybrids do not, of course, show any chromosome number heterozygosity and hence do not form trivalents at meiosis. The spermatogenesis of a single Yass × Murrumbateman F_1 male was entirely normal, which is not unexpected, since these localities are only about 12 miles apart. On the other hand, Hall × Wodonga hybrids (pair-mating 7, Table 1) show various minor cytological abnormalities. These localities are about 150 miles apart. The chiasma frequencies of the three Hall × Wodonga hybrids are definitely subnormal, although possibly less so than those of the Kyeamba × Collector hybrids. The fall in chiasma frequency is due in part to the *AB* and *EF* bivalents rather frequently having a chiasma in one arm only (Figs. 3(*a*), 4(*c*)); it is also due in part to instances of asynapsis on the part of one of the four small chromosome pairs and to the very occasional asynapsis of the *St*/*Bl CD* pair.

There accordingly seems to be a generally reduced synaptic affinity between the Hall and the Wodonga chromosomes in these hybrids. Whether it is always the same one of the four small pairs which is asynaptic in these individuals is uncertain, but seems probable (at any rate, we have never seen two of the small pairs asynaptic in the same cell). Assuming that it is always the same one, we have arbitrarily designated it as *4* in Figure 3.

Partially compensating for the deficiency of chiasmata in these individuals are certain anomalous associations between the ends of heterologous chromosomes. We believe these associations to be due to terminalized chiasmata, but since all observations were made on metaphase chromosomes there is no formal proof of this interpretation; however, we have assumed that these associations are chiasmata when calculating the chiasma frequencies and shall henceforth refer to them as such.

There seem to be at least five types of anomalous chiasmata:

(1) Between the proximal end—probably the short arm—of a chromosome *4* and one of the two arms of an *AB* chromosome (Figs. 3(*b*), 3(*e*), 3(*f*)).

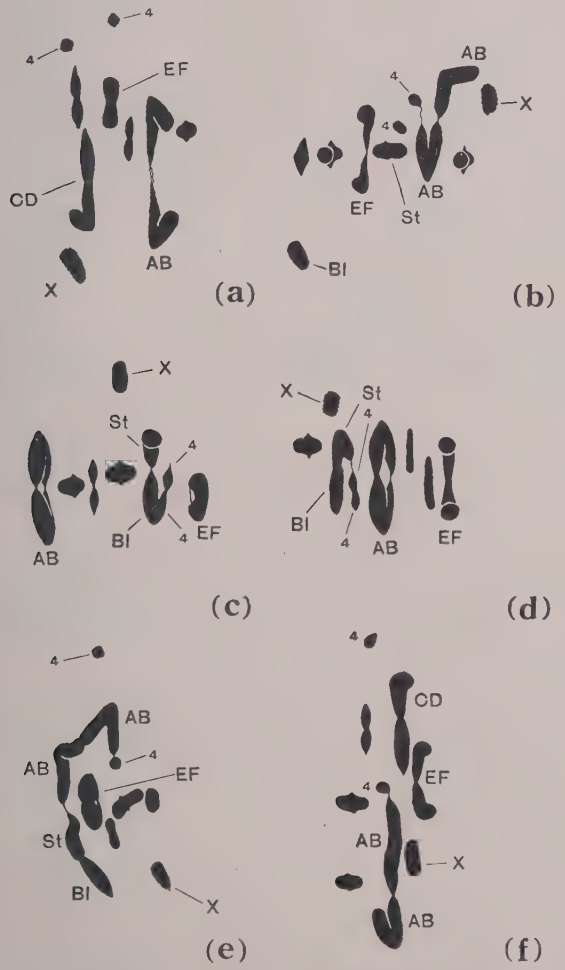

Fig. 3.—First metaphases in Hall × Wodonga hybrids (in side view). (*a*) From individual No. 1827, the remainder from No. 1825. These show various types of anomalous chiasmata described in the text. In (*a*) two univalent *4*th chromosomes can be seen, and in (*b*), (*e*), and (*f*) there are single univalent *4*th chromosomes.

(2) Between the same end of a chromosome *4* and the proximal end—probably the short arm—of a Blundell *CD* chromosome (Fig. 3(*c*)).

(3) Between the same end of a chromosome *4* and the tip of the shorter arm of a Standard *CD* element (Fig. 3(*d*)).

(4) Between one of the ends of an *AB* chromosome and the tip of the shorter arm of a Standard *CD* chromosome (Fig. 3(*e*)).

(5) Between the proximal end of a Blundell chromosome and the tip of the longer arm of an *EF* chromosome (Fig. 4(*c*)).

Thus, if we call the short arm of a Blundell chromosome I, that of a *4* chromosome II, the tip of either the *A* or the *B* limb (we do not know which) III, the tip of the shorter arm of a Standard *CD* IV, and the tip of the longer arm of a Standard *EF* V, then the following relationships seem to exist: II $\cong$ III, II $\cong$ I, II $\cong$ IV, III $\cong$ IV, I $\cong$ V. The remaining equivalences between I and III, I and IV, V and II, V and III, and V and IV presumably all exist, but have not been proved by direct observation. In these hybrids we know that the Standard *CD* comes from the Wodonga parent and the Blundell *CD* from the Hall parent, but we cannot distinguish the homologues of the cytologically homozygous pairs.

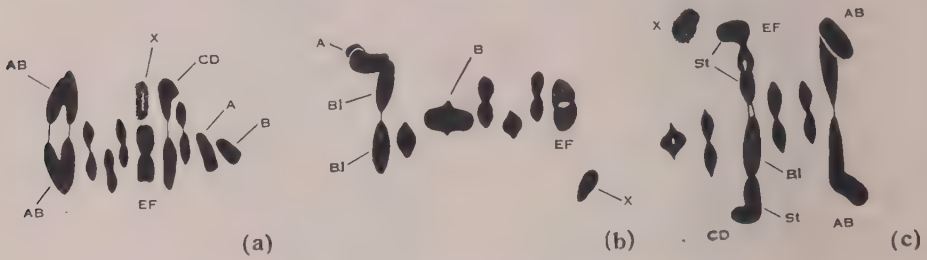

Fig. 4.—First metaphases (in side view) from various individuals. (*a*) Cell from natural dissociation heterozygote No. 1833 which (as a result of non-disjunction) contained two *AB* chromosomes, an *A*, and a *B*; (*b*) cell from an individual of the 17-chromosome race (Black Range locality) in which the *A* bivalent is paired with the proximal end of the *Bl*/*Bl* bivalent; (*c*) from Hall $\times$ Wodonga hybrid No. 1825, showing pairing between the Blundell chromosome and the long arm of an *EF* chromosome.

There has been in the past a disposition to interpret anomalous pairing between heterologous chromosomes, occurring in hybrid material, to heterozygosity for translocations, the parent forms being assumed to be cytologically homozygous. Thus Dobzhansky and Tan (1936), from observations on the salivary-gland chromosomes of *Drosophila pseudoobscura* $\times$ *D. miranda* hybrids, concluded that about five translocations had occurred since the evolutionary separation of those species. And Callan and Spurway (1951—see also Spurway 1953), having found some chains of four chromosomes at meiosis in interracial hybrids of *Triturus cristatus*, likewise supposed that these were heterozygous for translocations which had arisen during the evolutionary differentiation of the races.

Such an interpretation, even if applicable in the instances cited (which seems quite doubtful to us) does not fit the facts in the case of the Hall $\times$ Wodonga hybrids of *M. scurra*. There are too many different homologies, on any interpretation. Thus it seems likely that these individuals have a number of their chromosome tips more or less homologous to one another, having these regions reduplicated six, eight, or perhaps more times. There does not seem any compelling reason to suppose that this is due to their being interpopulation hybrids and we suppose that both parent populations are homozygous for these reduplicated chromosome ends. Thus it is probably only the incomplete synapsis of the hybrids which leaves certain of these

chromosome tips free to pair with one another, heterologously. Paradoxically, asynapsis seems to permit synaptic forces, which never get a chance to do so in non-hybrid material, to express themselves. It is of course, probable that homology between the tips of different chromosomes indicates a common origin (which implies translocation). But such translocations would not have to have occurred during the differentiation of the Hall and Wodonga populations from an ancestral one, and could have taken place at a much more remote period in phylogeny. Reduplication of chromosome tips (perhaps only the terminal one or two genetic loci) may be an inheritance from the earliest times in the history of the group and may be comparable to the "ectopic" pairing studied in the salivary-gland chromosomes of *Drosophila* by Slizynski (1945) and Kaufmann *et al.* (1948).

We have no reason to suppose that the asynapsis seen in the Hall × Wodonga hybrids is due to any heterozygosity for chromosomal rearrangements, and it seems more probable that it is "genic" in origin, being perhaps caused by different rates or degrees of spiralization in the chromosomes derived from different localities. Even though slightly developed, it could, in combination with the anomalous chiasmata, lead to a considerable degree of sterility. There can be little doubt, for example, that the interpopulation hybrids Nos. 1825 and 1827 would be less fertile than any of the interracial hybrids we have studied, except perhaps the Kyeamba × Collector ones, where there is also a considerable geographical distance between the parent populations.

V. Evidence as to how the *AB* Chromosome became Broken

Since the species which appear to be most closely related to *M. scurra* (an undescribed one from near Singleton, N.S.W., and the members of the genus *Keyacris*) all have a large metacentric chromosomes obviously homologous to the *AB* of the eastern race of *M. scurra*, we earlier assumed that the fused *AB* condition preceded the broken condition in the evolution of the species, rather than vice versa (White 1956). The 15-chromosome race, which is assumed to have given rise to the 17-chromosome one, occupies a considerably larger area at the present time and in some respects it shows greater cytogenetic variability (e.g. it includes cytologically polymorphic and—in Victoria—monomorphic populations; and in certain areas chromosome *CD* shows three alternative sequences instead of only two).

Such an interpretation, when first put forward, did, however, present serious difficulties. It involved the assumption that a metacentric element, presumably possessing a single centromere, had "dissociated" into two acrocentrics, each of which should have a centromere and two telomeres. Where could the extra centromere and two additional telomeres have come from? That the *A* and *B* elements of the western race do possess short arms (i.e. that they are not strictly telocentric) is shown by the fact that one at least of them occasionally shows a chiasma in the short arm.

The presence of the *St–Bl–A–AB–B* quinquevalents in approximately 2 per cent. of the spermatocytes of those hybrids which happen to be *St/Bl* heterozygotes seems to supply critical evidence as to the origin of the dissociation. Clearly the proximal end of the *A* element is homologous to the corresponding region of a *CD*

chromosome carrying the Blundell rearrangement. The exact length of the homologous segment cannot be determined, but it presumably includes the short arm and the centromere of both chromosomes.

Thus the "dissociation" was almost certainly a special type of translocation involving an *AB* and a Blundell-carrying *CD* chromosome, the centromere and short arm of the *A* chromosome (including the telomere) being derived from the Blundell element. The *B* acrocentric presumably retained the centromere of the original *AB*; there is no definite evidence as to where it acquired a telomere from, although it seems likely that this was provided by the distal end of the Blundell chromosome.

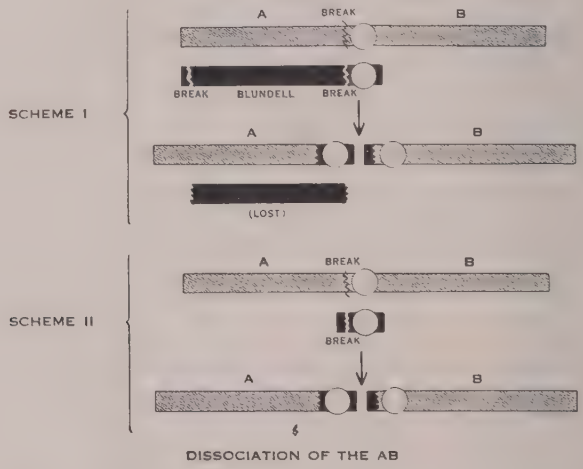

Fig. 5.—Diagram showing two schemes whereby the dissociation of the *AB* chromosome could have taken place (explanation in text).

We might suppose that the dissociation of the *AB* was a 3-break rearrangement in which the *AB* broke in the *A* limb, very close to the centromere, and the Blundell chromosome broke in two places, one close to the centromere and one close to the distal end (Fig. 5, scheme I). Alternatively, it is possible that the individual in which the dissociation occurred was already carrying, in addition to a pair of *CD* chromosomes (*Bl*/*Bl*, *St*/*Bl*, or *St St*) a Blundell chromosome from which the greater part of the longer arm had been deleted, i.e. a minute element with the required centromere and telomeres but very little else. In this case a simple 2-break translocation between such an element and the *AB* would have constituted the dissociation (Fig. 5, scheme II). Such a small extra chromosome fragment (not necessarily derived from a *CD* chromosome) was found in a single individual from Wombat, N.S.W. (out of 1000 examined); thus such fragments, although undoubtedly rare in *M. scurra*, are not necessarily lethal.

A third possible mechanism of dissociation, namely that the translocation involved a whole Blundell chromosome and that a deletion occurred subsequently, instead of simultaneously (Fig. 5, scheme I) or previously (Fig. 5, scheme II), seems

less probable, since it would have given rise to a double-length acrocentric which is hardly likely to have persisted until a convenient deletion reduced it to a normal length.

Regardless of whether the deletion occurred before, after, or at the same time as the translocation, the general mechanism of the dissociation seems obvious. We are necessarily led to the conclusion that the western race of *M. scurra* is tetrasomic for a minute region which is present at the proximal ends of the *A* chromosomes and is also represented in the *CD* pair. Probably it is also tetrasomic for the telomere and a minute region at the proximal end of the *B* chromosome, but it is not likely that any critical evidence on this point could be obtained unless we had an individual heterozygous for a distal inversion in the *CD* pair (in the same way that the proximal heterozygosity of the *St/Bl* pair reveals the tetrasomic region in the *A* element). Unfortunately, no such inversion is known to exist.

In individuals of the western race, which have the *A* chromosome in the homozygous condition, pairing between an *A* and a Blundell chromosome has only been observed once. That was in an individual from the Black Range, near Yass, which was *Bl/Bl*; a single cell (Fig. 4(*b*)) showed an association—presumably due to a chiasma—between the proximal ends of a Blundell and an *A* chromosome. Apart from such a rare accident, which may perhaps occur only once in tens of thousands of meiotic divisions, pairing between *A* and *Bl* is only to be expected when both are present in the heterozygous condition, the *A* being accompanied by an *AB* chromosome and the Blundell *CD* by a Standard *CD*. This is because in such individuals the minute trisomic regions will not be able to "find" a homologous region except by pairing between the short arms of the *A* and the Blundell *CD*. Theoretically, it should perhaps be possible for the proximal region of the *A* to pair with the centromere region in the middle of the Standard *CD* chromosome; but no such pairing has been observed and it may be that the orientation of the chromosomes at zygotene is such as to preclude it. In individuals with two Blundell chromosomes the proximal regions of these will, of course, normally pair together and hence will not be available for pairing with the proximal end of the *A*. Thus it is only *AB/A*, *B*; *St/Bl* individuals that can form quinquevalents.

Although the above interpretation is the simplest which fits the facts, we should not forget that the evidence of the Hall × Wodonga hybrids suggests that the proximal end of the Blundell chromosome is to some extent homologous to the proximal end of the *A*th chromosome, and that other homologies between the extreme ends of the chromosomes seem to exist in this species. Thus it is not impossible that some other chromosome such as the *A*th acted as a centromere-donor in the dissociation of the *AB* and that sufficient homology exists between the proximal end of that element and the Blundell chromosome to lead to the formation of quinquevalents involving Blundell in the interracial hybrids. But the relatively high frequency with which the Blundell and *A* elements are paired in the interracial hybrids and the fact that such an association has been seen even in a complete homozygote (Fig. 4(*b*)) makes it likely that the "donor" was actually a Blundell chromosome and not merely one whose proximal end had an undefined degree of homology with the tip of the short arm of a Blundell element.

An examination of 1049 first metaphases in the three "spontaneous" broken fused *AB* heterozygotes which happened to be *St Bl* (two from Komungla "B" and one from Paddy's River "B") did not reveal a single cell with a quinquevalent (Table 4). It is thus rather probable that the dissociations present in these individuals did not involve the Blundell chromosome but that some other chromosomes acted as centromere-donors in these instances of dissociation. More individuals of this type would be needed, however, to establish this point with certainty, on account of the fact that some *St Bl* interracial hybrids did not show quinquevalents, or only did so very rarely. Unfortunately, no more spontaneous broken fused *AB* heterozygotes were collected in 1956.

TABLE 4

CONFIGURATIONS IN NATURALLY OCCURRING *AB A, B* HETEROZYGOTES WITH THE *CD* PAIR *St Bl*

Individual No.	Locality	Year of Collection	*A–AB–B* Trivalent	Bivalent and Univalent	Other Configurations
1833	Komungla "B"	1955	898	5	1*
1834	Komungla "B"	1955	14	—	—
1835	Paddy's River "B"	1955	137	—	—
Total			1049	5	1

* This cell (illustrated in Fig. 4(*a*)) had two *AB* chromosomes, paired with one another, and univalent *A* and *B* elements. Obviously mitotic non-disjunction had occurred at a spermatogonial division.

VI. Phylogeny and Significance of the *CD* Chromosome Sequences

It was hoped that the cytology of the *Mol/St* heterozygotes encountered in the Bungendore population as a result of the "contamination" of that colony by Standard *CD* chromosomes (expt. (*d*)) would provide some evidence as to the nature and genetic significance of the Molonglo sequence. Two *St Mol* heterozygotes were, in fact, recovered from the Bungendore population and, in the meanwhile, some natural *St Mol* heterozygotes were found at the Taemas Bridge and Sutton localities.

Since technical difficulties make a study of the pachytene configurations unprofitable in this material, evidence as to the nature of the rearrangements which have occurred is necessarily indirect. But all observations on chiasma formation are consistent with the hypothesis that the three *CD* chromosome sequences are related to one another as pericentric inversions in the manner shown in Figure 6. However, it seems that suppression of chiasma formation, in the inverted region, is absolute in all heterozygous bivalents. Thus chiasmata between the two centromeres of heterozygous bivalents, such as were found in certain individuals of *Trimerotropis sparsa* from Beaver, Utah, U.S.A. (White and Morley 1955) have never been seen in *M. scurra*. Thus no gametes with deficiencies and duplications are produced by individuals of the *St/Bl*, *St/Mol*, or *Bl/Mol* types.

Mol Mol bivalents quite frequently form a chiasma in the short arm (Fig. 1(*c*)) whereas *Bl Bl* bivalents almost never do so. Thus, in the terminology of Figure 6,

we might say that the *ABC* region has quite a high chiasma frequency, when homozygous, while the *A* region by itself has hardly any chiasma frequency (only a few doubtful instances of short arm chiasmata in *Bl/Bl* bivalents have been seen in thousands of individuals of that constitution studied).

Bl/Mol bivalents invariably show a single chiasma in the *G–Q* region of Figure 6. On the other hand, *St/Mol* bivalents may show a chiasma in the *ABC* region (Fig. 1(*d*)). The "experimental" *St/Mol* heterozygotes in the Bungendore colony did so quite frequently, while the natural heterozygotes in the Taemas Bridge and Sutton colonies only did so rarely.

The chiasma frequency of the *ABC* region of *St/Mol* bivalents seems to prove that if Molonglo arose as a pericentric inversion, it must have done so from the Standard sequence rather than from Blundell.

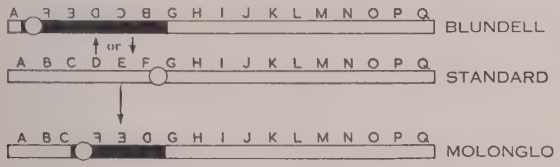

Fig. 6.—Diagram showing the most probable interpretation of the Standard, Blundell, and Molonglo sequences of the *CD* chromosome.

VII. Discussion

The cytological evidence completely confirms the view previously arrived at (White 1956) that the eastern (15-chromosome) and western (17-chromosome) races of *M. scurra* belong to the same species. The hybrids show no general histological or cytological abnormalities of spermatogenesis, such as are commonly found in species hybrids. Nor is there any evidence of genetic isolating mechanisms, except for a slight lowering of fertility in the F_1. Given time, these races might evolve into a pair of species differing in chromosome number—but they have as yet progressed only a very short distance along that road.

There are some rather considerable differences in various morphological characters (average size, length of the male subgenital plate, etc.) between the local colonies of *M. scurra*. But since these differences exist within each of the races, it is difficult to be certain whether there are any consistent morphological differences between them. In fact, it is quite possible that a taxonomic study based on external characters alone would divide the species into local geographic races which would not correspond at all to the cytological races.

The demonstration that the evolutionary dissociation of the *AB* chromosome was, in actuality, a type of translocation in which another chromosome (probably a *CD* chromosome carrying the Blundell sequence) donated its centromere, and probably also its telomeres, is of considerable theoretical importance. Many cytologists, faced with closely related races or species differing in chromosome number, have assumed that a process of simple "fragmentation" was responsible for an

evolutionary change from the lower to the higher number, without troubling to explain the increase in the number of centromeres. Such interpretations must now be decisively rejected (White 1957*a*). Other mechanisms may lead to increases in chromosome number in special cases. Thus Darlington and La Cour (1950), having found two apparently stable telocentric chromosomes paired with a metacentric in a Russian stock of the plant *Campanula persicifolia*, postulated that they had arisen through a process of "complementary misdivision" of the two centromeres at the same anaphase. While this mechanism is very likely the correct explanation of the particular telocentrics observed by these authors, it appears rather unlikely that it has played the important part in the evolution of chromosome numbers which they suppose. At any rate, it seems irrelevant to the present case. And it appears far more likely that the dissociations which have taken place in the coccinellid beetle *Chilocorus stigma* Say (Smith 1956) arose by the "*scurra* mechanism" than by complementary misdivision, as Smith has suggested. In fact, it seems likely that the *scurra* mechanism has functioned in the chromosomal evolution of many groups of animals. In many instances it is, of course, difficult to determine whether an evolutionary change in chromosome number has been from a higher to a lower number (by fusion) or vice versa (by dissociation). On general phylogenetic grounds we had already assumed (White 1956) that in *M. scurra* dissociation rather than fusion had occurred. And there is evidence that a number of different "dissociations" have occurred in other species of Morabinae. Thus although it still remains true that fusions can arise more easily than dissociations (in a group with both acrocentric and metacentric chromosomes), there seems no reason to deny that dissociations have played a significant role in the chromosomal evolution of many groups of animals. And in exceptional cases such as the mantids of the genus *Ameles*, studied by Wahrman (see Wahrman and O'Brien 1956) it may be that dissociations have been unusually frequent.

Considerable uncertainty has existed for many years as to the extent to which ordinary reciprocal translocations have played a part in the evolution of natural populations and species of animals (i.e. translocations where the breakage points are not adjacent to the centromeres or telomeres, as they are in the case of fusions and dissociations, which are also translocations of a kind).

Wright (1941) pointed out that the formation of aneuploid gametes by translocation heterozygotes would effectively prevent the establishment of translocations in meiotic populations of animals, except under very special circumstances. In general, the evidence both from studies of natural populations and from interspecific hybrids seems to support Wright's standpoint. On the other hand, several authors have claimed to have found heterozygosity for translocations in interspecific or interracial hybrids. Instances which have been, or could be, cited as apparent exceptions to Wright's conclusions include the *Drosophila pseudoobscura* × *D. miranda* hybrids (Dobzhansky and Tan 1936), the interracial *Triturus cristatus* hybrids (Callan and Spurway 1951; Spurway 1953) and the observations of Helwig (1955) on hybrids between the grasshoppers *Trimerotropis suffusa* and *Circotettix verruculatus* (whose assignment to different genera is probably unjustified). The chains of four chromosomes occasionally observed in non-hybrid individuals of the tettigoniid

Metrioptera brachyptera have also been claimed as evidence for translocation heterozygosity (White 1940).

We are now strongly inclined to interpret all these instances in the same way as we have done the Hall × Wodonga hybrids of *M. scurra*, i.e. to regard them as translocation homozygotes rather than heterozygotes. Or, to put it somewhat differently, we suppose that in each case we are dealing with small chromosomal regions which are duplicated in different members of the chromosome set, and for which both parent forms, as well as the hybrids, are consequently tetrasomic. In *T. cristatus*, *M. scurra*, the *Trimerotropis* × *Circotettix* hybrids, and in *M. brachyptera* the regions in question seem to be very minute segments at the tips of several chromosomes. The case of *D. pseudoobscura* and *D. miranda* is somewhat different, since the translocated or duplicated regions are interstitial.

That mutual translocations of minute terminal regions (a telomere plus perhaps 1–3 genetic loci) should be able to establish themselves from time to time in evolution is hardly surprising. And if they do so in most groups of organisms, it is not unexpected that there should be in many species some degree of homology between the tips of otherwise heterologous chromosomal elements.

Our search for cryptic cytological differentiation in *M. scurra* must be regarded as having been inconclusive. No definite evidence of paracentric inversions, for example, was found. The degree of asynapsis seems to increase in direct proportion to the geographic distance between the parental populations and, as far as we can tell from the small numbers of hybrids studied, regardless of whether they belong to the same chromosome-number race or not. But there is no evidence that this asynapsis is due to heterozygosity for structural rearrangements, and we are inclined to regard it as genic in origin. Helwig's (1955) assumption that asynapsis between the chromosomes of *T. suffusa* and *C. verruculatus* is mainly due to rearrangements likewise seems unwarranted, and leads to logical inconsistencies. The synaptic mechanism is so specific and precise that it is liable to be seriously upset in hybrids—probably by a variety of physiological and biochemical factors which have nothing to do with changes in gene sequence.

VIII. Acknowledgments

The laboratory hybrids were raised with skill and care by Mrs. M. Parker, who also prepared the sectioned material. Discussions with Dr. K. H. L. Key, Division of Entomology, C.S.I.R.O., have proved most fruitful in clarifying many of the issues raised by the present work.

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CYTOGENETICS OF THE GRASSHOPPER *MORABA SCURRA*

II. HETEROTIC SYSTEMS AND THEIR INTERACTION

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With a Statistical Appendix by B. Griffing†

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Summary

Moraba scurra Rehn shows inversion-polymorphism in two different chromosome pairs, "*CD*" and "*EF*". Each of these systems produces a heterotic effect on male viability. The cytological polymorphism of the *CD* pair is present in almost all populations except in the south-western part of the distribution area of the species (Tumut, N.S.W., to Merton, Vic.), where only the Standard sequence is present. Some populations have the Standard *CD* much more frequent than the Blundell *CD*, while in other localities the relationship is reversed and in five colonies a third sequence, Molonglo, was found, either replacing Standard or coexisting with Standard and Blundell.

The cytological polymorphism of the *EF* chromosome is distributed rather irregularly throughout the geographic area occupied by the species. Many populations have only the Standard type of *EF* chromosome and even in those which also contain the Tidbinbilla sequence the latter is always greatly outnumbered by Standard.

In populations containing both cytological polymorphisms, these are apparently not combined at random, there being a genetic interaction between them, as far as the viability of the males is concerned. This interaction leads to a deficiency of *Bl/Bl*, *St/St* individuals and to a further deficiency of genotypes in which Tidbinbilla coexists with the Standard *CD* chromosome, either in the heterozygous or the homozygous condition.

On the assumption that the overall selective values of these genotypes are generally similar to their effects on male viability, those populations which possess both heterotic mechanisms must have a complex balanced polymorphism, involving equilibria between the alternative gene sequences of the two different chromosome pairs, as well as between those of the same pair.

I. Introduction

In an earlier paper (White 1956), based on work carried out in 1955, it was concluded that the chromosomal rearrangements in the *CD* and *EF* chromosomes of *Moraba scurra* Rehn (Orthoptera: Eumastacidae) produce heterotic effects on male viability, since there is a systematic deficiency of cytological homozygotes (and a corresponding excess of heterozygotes) in the natural populations. It thus seems probable that each of these chromosomes possesses a system of true balanced polymorphism.

Certain questions remained, however, unanswered in that paper. Some of these were concerned with the genetic properties of the rare Molonglo sequence

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of the *CD* chromosome. A more important question was the meaning of the apparent correlation between the frequencies of the Blundell sequence of the *CD* chromosome and the Tidbinbilla sequence of the *EF* element, when one considers the cytogenetic constitution of 41 colonies of the species studied in 1955. Such a correlation, although formally significant statistically, could be more or less spurious. Alternatively, it was considered possible that it might indicate the existence of a genetic interaction between the two heterotic mechanisms; but the evidence obtained in 1955 did not establish the existence of an interaction of this type.

Collecting in 1956 was specifically directed towards solving some of these questions. Particular attention was directed towards colonies containing the Molonglo sequence. And several large samples were collected with the intention of determining whether, in fact, a genetic interaction between the two heterotic mechanisms exists or not. These samples naturally had to be selected for their suitability for this purpose since, for example, it would have been of little use to study colonies where (as at Michelago, N.S.W.) the frequency of the Tidbinbilla sequence is so low that an impossibly large number of individuals would have had to be analyzed to provide a test of the hypothesis. As a consequence of the correlation referred to above, the requirement that our "large" samples should contain a reasonably high frequency of the Tidbinbilla sequence automatically ensured that they were also "high Blundell" colonies.

We believe the identification of the chromosome types to be so simple in *M. scurra* that no misclassification of individuals can have occurred in the present work. A small percentage of individuals cannot be classified for lack of first metaphases in the testis. But these individuals are far too few in most samples to introduce any bias into the data—for example, in order to score 1000 individuals from Wombat, N.S.W., it was necessary to examine 1007.

We have already described (White 1956) the life cycle and ecology of this species of grasshopper, which is restricted to habitats of a special type that have by now largely disappeared as a result of grazing by sheep. Thus the colonies which have persisted are isolated relicts of what must have been a much more widespread and more continuous distribution a hundred years ago. Since *M. scurra* is apterous and its motility is very limited, migration between colonies is hardly possible at all at the present time and, even when the population was far more continuous, could not have played an important part in its population dynamics.

II. The Geographic Distribution of M. scurra

The location of the main colonies which we have studied is shown in Figures 1 and 2 of the present paper and in Figure 1 of White (1956). In some instances it has not been possible to show several localities situated very close together by separate symbols.

One may legitimately ask how far the distribution of *M. scurra* extends beyond the colonies we have studied. Can the colonies at Bong Bong, Orange, Michelago, Young, and Merton be regarded as peripheral? In some areas we believe that sufficient collecting has been carried out to define the distributional limit of the species approximately. But in other areas it is probable that more collecting would extend

the known range greatly. Braidwood and Bong Bong are almost certainly limital on the east. Between these localities and the coast there extends dense forest entirely unsuited to *M. scurra*. Northward, it is quite possible that the species occurs between Bong Bong and Orange. However, we failed to find it in the cemeteries at Wellington, Molong, Mudgee, and Uralla, so it is unlikely that it extends far north of Orange. To the west, it is not probable that the 17-chromosome race exists far to the west of Young and Cootamundra, since we failed to collect it at Forbes, Grenfell, Stockinbingal, and Temora cemeteries. Further south, however, it may extend into the area between Wagga Wagga and Albury. The largest area which appears suitable for the species, and in which no collecting has been carried out, is from Batlow and Michelago southward to Delegate and from there westward through Victoria to Benalla and Merton. Although necessarily absent from the higher regions of the Snowy Mountains it may well occur at lower elevations throughout a considerable part of that area.

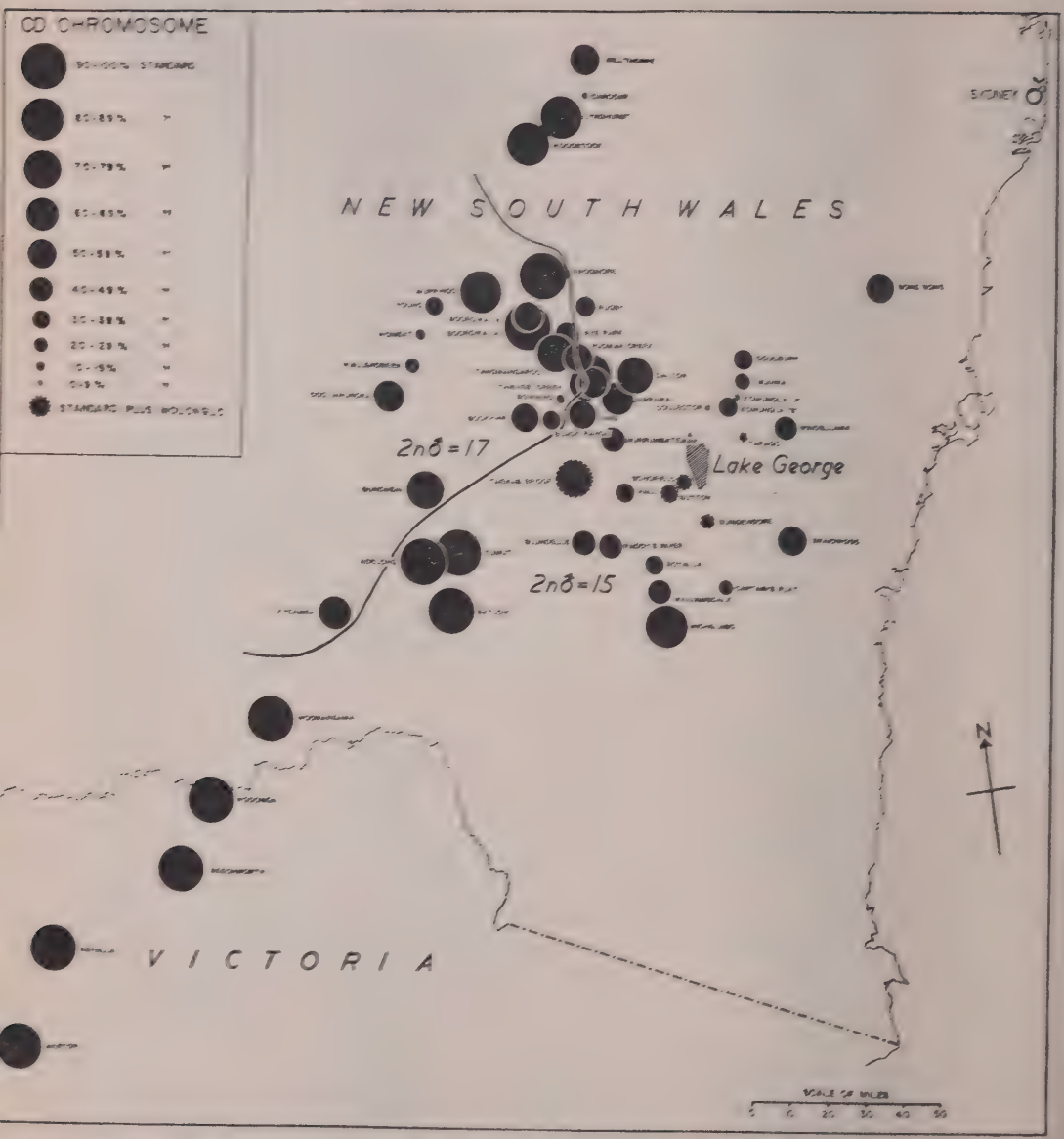
The distribution of *M. scurra* overlaps those of at least three other species of Morabinae. In the eastern part of its range there is a broad overlap with *M. amiculi*, which has been collected a few miles east of Captain's Flat, near Marulan, in the Rye Park cemetery, and on Mt. Canobolas, near Orange. Since this species inhabits areas of dry sclerophyll forest, usually on ridges, it is unlikely to coexist with *M. scurra* at any locality, and direct competition of these species seems out of the question. Two other Morabinae do, however, definitely coexist with *M. scurra*. One is a species of *Keyacris* which was taken with *M. scurra* in the cemetery at Millthorpe. The main area of distribution of this species is northward from Millthorpe, so that the geographic overlap with *M. scurra* must be small.

The other species which has been collected with *M. scurra* is an undescribed member of the *cultrata* group of *Moraba* which is only known from the Taemas Bridge locality. Here, as at Millthorpe, the two species of morabines form a mixed colony, on the same *Helichrysum* shrubs. It seems quite possible that the distributions of *M. scurra* and *M. viatica* overlap somewhere in Victoria, but this is unproven.

M. scurra does not, in general, seem to show diminution of chromosomal heterozygosity at the periphery of its range. The marginal colonies at Bong Bong, Millthorpe, Wombat, and Kyeamba all show a fairly high level of inversion heterozygosity. Only in the south-western part of its distribution, from Tumut and Batlow to Beechworth, Benalla, and Merton, do we find an absolutely or almost completely homozygous race. Some of these localities may lie close to the edge of the species distribution, but it does not seem possible that Tumut and Batlow are peripheral. Thus all we can say is that the levels of chromosomal heterozygosity are high throughout most of the Southern Tableland and western slopes, but probably lower in the Lake George region (Bungendore, Sutton, Tarago, Collector) and close to zero from the Tumut–Batlow area southward into Victoria.

III. Geographic Distribution of the Chromosome Sequences

The basic data on the distribution of the chromosome sequences is contained in Table 1 of White (1956) and in Tables 1, 2, and 3 of the present paper. The situation, as far as the *CD* chromosome is concerned, is shown graphically in Figure 1,



g. 1.—Map of the distribution of *M. scurra*, showing the frequency of the Standard type of *CD* chromosome in e various colonies discussed in the text or the combined frequency of the Standard and Molonglo sequences in e case of those colonies where the latter is present. Some localities geographically near together have been grouped the manner shown in Table 4. Relatively little significance should be attached to the symbols for Milthorpe, cgmore, Pingip, Pullman Creek, Coonamnora, Jerrawa, Braidwood, Kyeamba, Woomargama, and Merton, hich are each based on less than 10 individuals. Orange, the most northerly locality at which *M. scurra* has been ound, is not shown since only one male was collected. Sydney and Lake George are included only for purposes of orientation and are not localities for *M. scurra*.

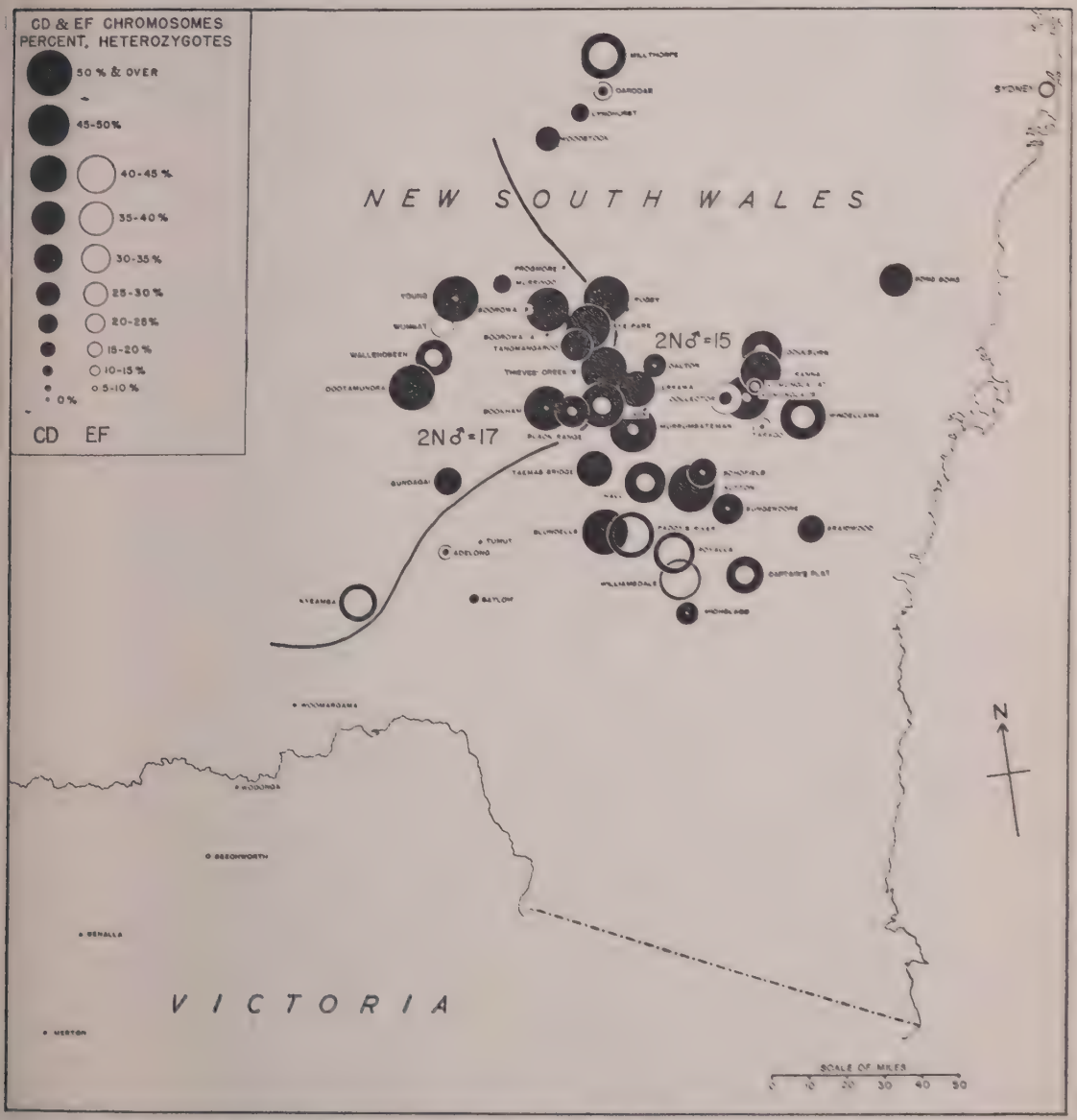


Fig. 2.- Map showing the extent of heterozygosity for the *CD* and *EF* chromosome pairs in the various colonies discussed in the text (data in Table 4). All-black symbols represent colonies where the Tidbinbilla sequence is not known to occur; black symbols with a white centre those where the percentage of heterozygosity for the *CD* pair exceeds that for the *EF* pair; and white symbols with a black centre the four colonies where that relationship is reversed. At Wombat the percentage heterozygosity for the two chromosome pairs is almost exactly equal. As in Figure 1, neighbouring localities have been grouped in some instances. The symbol for Boorowa "A" is shown about 5 miles south of its true position, to avoid overlapping with Boorowa "B". As in Figure 1, relatively little significance should be attached to the samples which are very small. It is, for example, entirely possible that the Tidbinbilla sequence occurs at such localities as Braidwood and Cootamundra.

where the size of the black circles represents the frequency of the Standard type of *CD* chromosome. The data on which this figure is based are given in Table 4.

It will be seen that there is a rather large area, which includes Tumut, Batlow, Adelong, Woomargama, and the four localities in Victoria, within which the Blundell sequence is either extremely rare or absent altogether, the *CD* chromosome being

TABLE 1

COMPOSITION OF THE SIX "LARGE" ADULT MALE SAMPLES

Chromosome *EF*	Locality	Year of Collection	Chromosome *CD*			Totals
			St/St	*St Bl*	*Bl Bl*	
St/St	Wombat*	1956	14	197	509	720
	Hall*	1955	52	256	250	558
	Hall*	1956	46	221	201	468
	Royalla "A"	1955	42	184	167	393
	Royalla "B"	1956	37	186	156	379
	Williamsdale	1956	39	78	45	162
St Tid	Wombat*	1956	5	61	195	261
	Hall*	1955	22	87	98	207
	Hall*	1956	7	50	63	120
	Royalla "A"	1955	25	117	136	278
	Royalla "B"	1956	16	96	86	198
	Williamsdale	1956	21	56	46	123
Tid/Tid	Wombat*	1956	0	8	11	19
	Hall*	1955	0	2	10	12
	Hall*	1956	0	5	7	12
	Royalla "A"	1955	3	12	14	29
	Royalla "B"	1956	2	8	13	23
	Williamsdale	1956	3	7	5	15
Totals	Wombat*	1956	19	266	715	1000
	Hall*	1955	74	345	358	777
	Hall*	1956	53	276	271	600
	Royalla "A"	1955	70	313	317	700
	Royalla "B"	1956	55	290	255	600
	Williamsdale	1956	63	141	96	300

* Cemetery samples.

almost invariably of the Standard type. Within this same area the Tidbinbilla sequence of the *EF* chromosome is likewise absent or very rare, so that the populations are almost or completely monomorphic cytologically. On the other hand, the populations to the north of this area, i.e. those of the Southern Tableland and western slopes of New South Wales, almost all show a considerable degree of cytological polymorphism for the *CD* chromosome.

The Blundell sequence of the CD chromosome reaches high frequencies in the Lake George area and from there northward as far as Goulburn and south-west to Captain's Flat. In the far north of the range of the species a colony at Tamworth cemetery showed a very high frequency of Blundell. In the western CD-chromosome race of M. scurra most populations show a rather low frequency of Blundell, but this is not true of an area around [illegible] and another (or perhaps an extension of the same one) around Young, Wombat, and [illegible]. The very low frequency of Blundell at Wallendbeen is remarkable; this colony may possibly represent a stage in a cline leading to the almost or completely monomorphic populations of the Tumut–Batlow area and northern Victoria.

The third known sequence of the CD chromosome is a rare one which has been named Michelago. Its probable relationship to the common, Standard and Blundell sequences has been discussed already (White 1957). Michelago was first encountered 5 miles S. of Bungendore, where the Standard CD is absent. Subsequently, the Towrang Bridge colony was found to contain Michelago in a closely similar sequence (White [illegible]). In [illegible] this colony was visited rather late when [illegible], so that it was difficult to distinguish between Blundell and Michelago chromosomes and no identification was possible. In [illegible] the colony was visited earlier in the season, with the results given in Table 3, from which the frequencies of the three sequences can be calculated as: Michelago = [illegible], Blundell = [illegible], Standard = [illegible]. Obviously, in this population all three coexist satisfactorily.

In [illegible] three other colonies containing Michelago were discovered. At one of these, [illegible] miles N. of Tarago, N.S.W., Blundell and Michelago were present, but the Standard CD was absent, as in the case of the Bungendore colony. It may be objected that the sample of [illegible] individuals was hardly large enough to exclude the possibility that the Standard CD might be present in low frequency at the Tarago locality. However, this locality is only about a mile from Lake Bathurst cemetery, with which it is connected by a strip of ecologically suitable land along the railway, so that it is probably legitimate to add the [illegible] individuals from the cemetery (all Blundell/Blundell) to the [illegible] from the Tarago locality. There is a strong probability that the population of this area is of the same type as the Bungendore colony, although with an even lower percentage of Michelago chromosomes.

The Yarra and Schofield colonies seem to be very similar in composition. Both contain all three of the CD chromosome sequences, but the frequency of Michelago is much lower at Schofield than at any of the other localities where it occurs (Fig. 1).

In summary, it may be stated that Michelago appears to have displaced Standard in the Bungendore and Lake Bathurst areas (these being the only colonies known which lack the Standard sequence), while at Schofield, Yarra, and Towrang Bridge all three sequences seem to coexist in stable equilibrium. Thus we believe that the displacement of Standard by Michelago at Bungendore and Tarago was an [illegible], i.e. Michelago has adaptively replaced Standard, perhaps because they have similar genetic properties. With the exception of the Towrang Bridge colony, Michelago seems to be confined to a rather restricted area in the Lake George region. But even within that area it is apparently absent from such localities as [illegible], the two [illegible] colonies, and Tarana.

Three of the localities at which Molonglo occurs possess the Tidbinbilla *EF* sequence in very low frequency, while at Sutton and Taemas Bridge Tidbinbilla is completely absent. If, as will be suggested later, the frequency of Tidbinbilla is kept down to a low level in many colonies by a high frequency of Standard *CD* (and is absent altogether from many localities for this reason), then (since Standard *CD* was absent at Bungendore and Tarago) we might conclude that the Molonglo sequence is perhaps even more effective than the Standard one in keeping down the frequency of Tidbinbilla.

TABLE 2

COMPOSITION OF 15 "SMALL" ADULT MALE SAMPLES

Chromosome *EF*	Locality	Year of Collection	Chromosome *CD*			Totals
			St/St	*St/Bl*	*Bl/Bl*	
St/St	Michelago*	1955	273	111	6	390
	Michelago*	1956	96	42	5	143
	Murrumbateman*	1955	25	86	55	166
	Black Range "B"	1956	6	19	23	48
	Komungla "B"	1955	13	81	63	157
	Goulburn	1955	17	57	58	132
	Collector	Apr. 1955	1	24	80	105
	Collector	Oct.–Nov. 1955	0	9	92	101
	Paddy's River "A"	1955	8	29	20	57
	Paddy's River "B"	1955	30	64	27	121
	Captain's Flat*	1955	3	14	19	36
	Young*	1955	8	27	22	57
	Bowning "A"	1955	0	15	28	43
	Windellama "B"	1955	7	14	14	35
	Wallendbeen*	1956	10	48	63	121
St/Tid	Michelago*	1955	6	4	0	10
	Michelago*	1956	4	3	0	7
	Murrumbateman*	1955	1	18	12	31
	Black Range "B"	1956	1	2	5	8
	Komungla "B"	1955	3	9	9	21
	Goulburn	1955	2	17	13	32
	Collector	Apr. 1955	1	13	60	74
	Collector	Oct.–Nov. 1955	0	15	70	85
	Paddy's River "A"	1955	4	22	14	40
	Paddy's River "B"	1955	9	39	25	73
	Captain's Flat*	1955	0	5	7	12
	Young*	1955	0	4	1	5
	Bowning "A"	1955	0	3	7	10
	Windellama "B"	1955	0	8	6	14
	Wallendbeen*	1956	2	14	20	36

TABLE 2 (*Continued*)

Chromosome *EF*	Locality	Year of Collection	Chromosome *CD*			Totals
			St/St	*St/Bl*	*Bl/Bl*	
Tid/Tid	Michelago*	1955	0	0	0	0
	Michelago*	1956	0	0	0	0
	Murrumbateman*	1955	0	2	1	3
	Black Range "B"	1956	0	0	0	0
	Komungla "B"	1955	0	0	0	0
	Goulburn	1955	0	1	0	1
	Collector	Apr. 1955	0	0	21	21
	Collector	Oct.–Nov. 1955	0	2	12	14
	Paddy's River "A"	1955	1	3	0	4
	Paddy's River "B"	1955	1	2	3	6
	Captain's Flat*	1955	0	2	1	3
	Young*	1955	0	0	0	0
	Bowning "A"	1955	0	0	1	1
	Windellama "B"	1955	0	1	2	3
	Wallendbeen*	1956	1	2	0	3
Totals	Michelago*	1955	279	115	6	400
	Michelago*	1956	100	45	5	150
	Murrumbateman*	1955	26	106	68	200
	Black Range "B"	1956	7	21	28	56
	Komungla "B"	1955	16	90	72	178
	Goulburn	1955	19	75	71	165
	Collector	Apr. 1955	2	37	161	200
	Collector	Oct.–Nov. 1955	0	26	174	200
	Paddy's River "A"	1955	13	54	34	101
	Paddy's River "B"	1955	40	105	55	200
	Captain's Flat*	1955	3	21	27	51
	Young*	1955	8	31	23	62
	Bowning "A"	1955	0	18	36	54
	Windellama "B"	1955	7	23	22	52
	Wallendbeen*	1956	13	64	83	160

* Cemetery samples.

IV. The Evidence for Heterotic Effects on Viability

It is now rather generally accepted that polymorphism for chromosomal inversions in natural populations usually owes its preservation to heterosis, i.e. to the heterozygotes having a higher selective value than either homozygous genotype. Nevertheless, this idea has not been without its critics, or at any rate there is no unanimity as to the adaptive significance of heterosis of this type.

As far as *M. scurra* is concerned, we have already published some evidence that each of the two cytological polymorphisms produces a heterotic effect on male

viability, the natural populations showing a slight but significant deficiency of homozygotes and a corresponding excess of heterozygotes, by comparison with the theoretical expectation (White 1956). To that evidence we may now add the information derived from the 1956 collections. The final picture, as calculated by the method of Levene (Dobzhansky and Levene 1948; Levene 1949), is given in Tables 5 and 6. Among the seven large samples (of over 300 individuals) only one (Royalla "B") shows a significant deficiency of *CD* homozygotes and one (Royalla "A") shows a significant deficiency of *EF* homozygotes. However, in the case of each chromosome pair, six out of the seven samples do show a deficiency of homozygotes and the overall deficiency is in each case quite significant by Levene's *t*-test. If we add the data from the small samples (Table 6) we obtain an even more highly significant negative *t* value for the *CD* chromosome, although the negative *t* for the *EF* chromo-

TABLE 3

ADULT MALE SAMPLES COLLECTED IN 1956 BUT NOT PREVIOUSLY INCLUDED IN TABLES 1 AND 2

Localities	Chromosome *CD*						Chromosome *EF*			Totals
	Mol Mol	*St Mol*	*Bl Mol*	*St St*	*St Bl*	*Bl Bl*	*St St*	*St Tid*	*Tid Tid*	
15-chromosome race										
Sutton	—	3	15	12	50	53	133	—	—	133
Schofield	—	—	2	8	25	49	78	5	1	84
Taemas Bridge	—	14	4	97	71	14	200	—	—	200
Bungendore	3	2‡	12	—	1‡	13	30	1	—	31
Tarago, 3 miles N. of	—	—	5	—	—	35	34	6	—	40
Lake Bathurst*	—	—	—	—	—	18	11	7	—	18
Windellama*	—	—	—	2	15	1	13	4	1	18
Collector*	—	—	—	—	4	6	8	2	—	10
Goulburn*	—	—	—	3	3	1	6	1	—	7
Bong Bong*	—	—	—	6	6	5	17	—	—	17
Murrumbateman*	—	—	—	2	13	9	23	1	—	24
Tumut*	—	—	—	17	—	—	17	—	—	17
Batlow*	—	—	—	17	3	—	20	—	—	20
Woomargama	—	—	—	9	—	—	9	—	—	9
Beechworth, Vic.*	—	—	—	17	—	—	15	2	—	17
Merton, Vic.*	—	—	—	4	—	—	4	—	—	4
Royalla "A"	—	—	—	1	15	9	13	11	1	25
Pudman Creek	—	—	—	3	1	2	6	—	—	6
Yass, 10 miles NNE. of	—	—	—	4	13	1	12	5	1	18
Yass, 10·9 miles N. of	—	—	—	9	5	—	14	—	—	14
Thieves' Creek "A"	—	—	—	8§	4§	1	13	—	—	13
Rugby, 2 miles SE. of	—	—	—	1	4	3	8	—	—	8
Lyndhurst*	—	—	—	11	3	—	14	—	—	14
Dalton, 6 miles W. of †	—	—	—	1	—	—	1	—	—	1

TABLE 3 (*Continued*)

Localities	Chromosome *CD*						Chromosome *EF*			Totals
	Mol/Mol	*St/Mol*	*Bl/Mol*	*St/St*	*St/Bl*	*Bl/Bl*	*St/St*	*St/Tid*	*Tid/Tid*	
17-chromosome race										
Bookham, 4 miles SE. of	—	—	—	—	2	7	7	2	—	9
Bowning, 2 miles ESE. of	—	—	—	—	1	5	4	2	—	6
Yass, 9 miles N. of	—	—	—	24	36	5	65	—	—	65
Yass, 9·2 miles N. of	—	—	—	9	8	5	17	—	—	17
Yass, 9·5 miles N. of	—	—	—	1	3	—	4	—	—	4
Thieves' Creek "B"	—	—	—	3	3	5	11	—	—	11
Rye Park, 6 miles S. of	—	—	—	—	4	4	8	—	—	8
Rye Park, 1·5 miles NNE. of	—	—	—	1	2	3	6	—	—	6
Yass, 6 miles NNW. of	—	—	—	—	2	4	4	2	—	6

* Cemetery populations.

† Collected in 1955, but not previously recorded.

‡ F_1 individuals from Paddy's River males introduced in 1955.

§ Including, in each case, two females.

some is slightly smaller. By comparing Table 6 of the present paper with Table 2 of White (1956) it will be seen that a further season's collecting has strengthened the evidence for a consistently heterotic effect on male viability—markedly so in the case of the *CD* chromosome and less strongly as far as the *EF* element is concerned.

The present data may be compared with those of Dobzhansky and Levene (1948) and Epling, Mitchell, and Mattoni (1953, 1955) on *Drosophila pseudoobscura*, which are among the few studies of this type where the numbers of individuals examined cytologically have been adequate to base interpretations on. Some important differences between the *Drosophila* material and our own are (1) that the *D. pseudoobscura* inversions undergo seasonal changes in frequency, no doubt related to the changing environment of the seasons, and (2) that most of the colonies with which Dobzhansky and Levene and Epling *et al.* were concerned contained several (up to five) different third-chromosome sequences, instead of just two alternatives (Standard and Blundell for the *CD* chromosome and Standard and Tidbinbilla for the *EF*) as is usual in *M. scurra*.

Dobzhansky and Levene found evidence of a systematic deficiency of inversion homozygotes in the natural populations of *D. pseudoobscura* studied by them and concluded that inversion heterozygosity leads to heterotic effects on viability in

TABLE 4

PERCENTAGE OF STANDARD *CD* CHROMOSOMES AND PER CENT. HETEROZYGOTES FOR THE *CD* AND *EF* CHROMOSOME PAIRS (DATA ON WHICH FIGURES 1 AND 2 ARE BASED)

Localities*	Year of Collection	No. of Individuals	Per Cent. Standard *CD* Chromosomes	Per Cent. Heterozygotes for:	
				CD Pair†	*EF* Pair†
Millthorpe	1955	6	50·0	66·7	33·3
Carcoar	1955	14	7·1	14·3	21·4
Lyndhurst	1956	14	89·2	21·4	—
Woodstock	1955	12	87·5	25·0	—
Bong Bong	1956	17	52·9	35·3	—
Frogmore	1955	3	100·0	—	—
Young	1955	62	37·9	50·0	8·1
Murringo	1955	104	88·0	24·0	—
Boorowa "B"	1955	44	54·5	63·6	—
Boorowa "A"	1955	60	100·0	—	—
Rugby	1956	8	37·5	50·0	—
Rye Park, 3 colonies	1955–56	30	45·0	50·0	—
Wombat	1956	1000	15·2	26·6	26·1
Tangmangaroo	1955	11	72·7	36·4	—
Pudman Creek	1955–56	7	57·1	28·6	—
Yass, 10·9 miles N. of Thieves' Creek "B" and 9, 9·2, and 9·5 miles N. of Yass	1956	14 97	82·1 63·9	35·7 51·5	—
Yass, 10 miles NNE. of	1956	18	58·3	72·2	27·8
Dalton "A" and "B"	1955	14	78·6	28·6	7·1
Goulburn	1955	165	34·2	45·5	19·4
Wallendbeen	1955–56	182	27·5	40·7	3·8
Cootamundra	1955	4	62·5	75·0	—
Bowning, 3 colonies	1955–56	67	19·4	32·8	22·4
Jerrawa	1955	5	60·0	40·0	—
Tiranna	1955	17	29·4	47·1	—
Komungla "A"	1955	26	7·7	15·4	11·5
Komungla "B"	1955	178	34·3	50·6	11·8
Collector	1955	400	8·4	15·8	39·8
Bookham, 2 colonies	1955–56	31	54·8	51·6	6·4
Black Range	1955–56	61	31·1	36·1	14·4
Yass	1955	24	52·1	70·8	20·8
Windellama "A" and "B"	1955–56	94	41·0	56·4	25·5
Murrumbateman	1955–56	224	39·1	53·1	14·3
Tarago and Lake Bathurst	1956	58	4·3‡	8·6	22·4
Gundagai	1955	16	78·1	31·3	—
Taemas Bridge	1956 only	200	74·3§	44·5	—
Hall	1955–56	1377	31·8	45·1	23·7
Sutton	1956	133	35·7§	51·1	—
Schofield	1956	84	25·6§	32·1	6·0
Bungendore	1955 only	200	25·8‡	39·5	3·5
Braidwood	1955	3	50·0	33·3	—
Adelong	1955	11	95·5	9·1	18·2
Tumut	1956	17	100·0	—	—

TABLE 4 (*Continued*)

Localities*	Year of Collection	No. of Individuals	Per Cent. Standard *CD* Chromosomes	Per Cent. Heterozygotes for:	
				CD Pair†	*EF* Pair†
Blundell's	1955	52	49·0	55·8	—
Paddy's River "A" and "B"	1954–55	366	43·6	51·6	41·3
Royalla "A" and "B"	1955–56	1325	32·8	46·6	36·8
Kyeamba	1955	9	66·7	44·4	33·3
Batlow	1956	20	92·5	15·0	—
Williamsdale	1956	300	44·5	47·0	41·0
Captain's Flat	1955	51	26·5	41·2	23·5
Michelago	1955–56	550	83·5	29·1	3·1
Woomargama	1956	9	100·0	—	—
Wodonga	1955	22	100·0	—	—
Beechworth	1956	17	100·0	—	11·8
Benalla	1955	17	100·0	—	—
Merton	1956	4	100·0	—	—

* In geographical order, from north to south.

† These are the observed percentages of heterozygotes, not those expected on the Hardy-Weinberg ratio.

‡ Molonglo, not Standard.

§ Molonglo plus Standard.

this species. The total number of individuals included in their data was 2319 and for the data as a whole they obtained $t = -6{\cdot}845$, $P < 10^{-6}$. However, using considerably larger numbers (total 7243) of the same species, trapped in the wild, Epling *et al.* (1955) did not obtain any consistent deficiency of homozygotes. In fact, there was a doubtfully significant deficiency ($t = -1{\cdot}63$, $P = 0{\cdot}05$) over the first 6 months of the year and a rather more significant excess ($t = +1{\cdot}77$, $P = 0{\cdot}035$) during July–December. The reason for the discrepancy between these two sets of data is far from clear and one can only hope that it will be resolved by future work.

In the case of *M. scurra*, the situation seems much clearer than in *D. pseudoobscura*, perhaps because there are fewer different inversion sequences and because there is only a single generation a year, so that the population does not have to face radically different environments in successive generations (climatic variation from year to year will certainly be less drastic than seasonal changes in temperate latitudes). The possibility has also to be faced that the trapping techniques of the *Drosophila* workers may not yield random samples of the populations being studied. Whatever other effects they may give rise to, and regardless of subsidiary questions such as the extent to which the environment is geographically divided into different ecological niches, the cytological polymorphisms associated with the *CD* and *EF* chromosomes of *M. scurra* clearly do give rise to heterotic effects on viability. The deficiency of homozygotes is relatively small (only a few per cent.) but, in spite of variations due to sampling error, is rather consistently present. We shall, however, show in the next

section that, superimposed on the heterotic effects of the two polymorphisms there is a genetic interaction between them, in those colonies of the species where they coexist. This considerably complicates the interpretation of the adaptive cytological polymorphism of *M. scurra* and seems to be a novel type of effect, not hitherto observed in the population genetics of *Drosophila* and only doubtfully analogous to some phenomena known in human genetics.

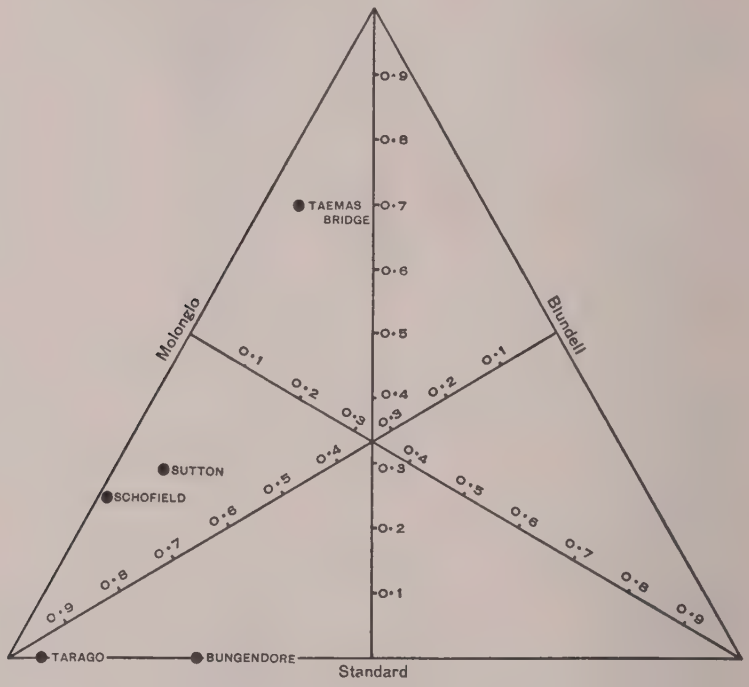


Fig. 3.—The frequencies of the *CD* chromosome sequences in the five populations in which Molonglo occurs.

V. Genetic Interaction between the Two Heterotic Systems

A colony of *M. scurra* which contains two *CD* chromosome sequences (Standard (*St*) and Blundell (*Bl*)) and two *EF* sequences (Standard and Tidbinbilla (*Tid*)) contains nine different karyotypes which can be arranged in a 3×3 table. To test whether the three *CD* chromosome pairs (*St*/*St*, *St*/*Bl*, and *Bl*/*Bl*) are randomly combined with the three *EF* ones (*St*/*St*, *St*/*Tid*, and *Tid*/*Tid*) we can calculate the expectation for each of the nine cells of the table from the marginal totals (e.g. in the case of the sample from Wombat, in Table 1, the expectation for *St*/*St*, *St*/*St* individuals is $19 \times 720/1000$ or 13·68). If the deviations from expectation, calculated in this way, proved to be generally non-significant, we should conclude that no genetic interaction between the two heterotic polymorphisms exists. It should be pointed out that in a table of this kind the deviations in each row and column necessarily sum to zero, and that the table as a whole has 4 degrees of freedom.

The data for the six large samples are shown in Table 1 and the deviations from expectation are given in Table 7. It must be admitted that in none of these

TABLE 5

LEVENE TEST ON LARGE SAMPLES

Chromosome	Locality	Year of Collection	h-H	σ^2	t	P	N
CD	Hall	1955	−8·18544	145·85074	−0·678	n.s.	777
	Hall	1956	−15·38616	113·04759	−1·447	n.s.	600
	Royalla "A"	1955	−6·35883	134·17635	−0·549	n.s.	700
	Royalla "B"	1956	−23·11093	118·56790	−2·122	0·017	600
	Wombat	1956	−8·07904	66·36506	−0·992	n.s.	1000
	Williamsdale	1956	+7·43239	73·31200	+0·868	n.s.	300
	Michelago	1955	−8·02753	28·44755	−1·505	n.s.	400
			−61·71554	679·76719	−2·367	0·009	4377
EF	Hall	1955	−10·21120	49·68312	−1·449	n.s.	777
	Hall	1956	+6·82569	26·66358	+1·322	n.s.	600
	Royalla "A"	1955	−22·45747	93·12323	−2·327	0·010	700
	Royalla "B"	1956	−3·45121	62·91539	−0·435	n.s.	600
	Wombat	1956	−6·57328	64·57561	−0·818	n.s.	1000
	Williamsdale	1956	−8·82471	43·28956	−1·341	n.s.	300
	Michelago	1955	−0·11264	0·20649	−0·248	n.s.	400
			−44·80482	340·45698	−2·428	0·008	4377

samples, considered individually, are the deviations large enough to be significant by a χ^2 test. On the other hand, it is apparent from an inspection of Table 7 that the

TABLE 6

LEVENE TEST ON WHOLE DATA (1955 AND 1956 COLLECTIONS)

Chromosome	Deviations	σ^2	t	P
CD (75 samples, totalling 7768 individuals)	Sum of 51 negative deviations = −167·82327 Sum of 21 positive deviations = + 36·43184 (3 samples showed no deviation) −131·39143	1208·26123	−3·780	0·00008
EF (47 samples, totalling 6906 individuals)	Sum of 30 negative deviations = −81·06646 Sum of 13 positive deviations = +33·23852 (4 samples showed no deviation) − 47·82794	509·33525	−2·119	0·01704

deviations are remarkably consistent from sample to sample, i.e. that both the sign and the relative magnitude of the deviations tend to be the same, cell for cell, in the

different samples. This consistency seems greater than could occur by chance, although it is not obvious just how its significance can be estimated statistically. It is not appropriate to use a χ^2 test over the data as a whole because, for one thing, this neglects the signs of the individual deviations. In Table 7 the summed deviations for 15 small samples have been added to the individual deviations for the six

TABLE 7

DEVIATIONS FROM EXPECTATION, CALCULATED FROM MARGINAL TOTALS OF TABLES 1 AND 2

Chromosome *EF*	Locality	Year of Collection	Chromosome *CD*		
			St/St	*St/Bl*	*Bl/Bl*
St/St	Wombat	1956	+0·32	+5·48	−5·80
	Hall	1955	−1·14	+8·24	−7·10
	Hall	1956	+4·66	+5·72	−10·38
	Royalla "A"	1955	+2·70	+8·27	−10·97
	Royalla "B"	1956	+2·26	+2·82	−5·08
	Williamsdale	1956	+4·98	+1·86	−6·84
	15 small samples (total)		+16·15	−8·48	−7·67
			+29·93	+23·91	−53·84
St/Tid	Wombat	1956	+0·04	−8·43	+8·39
	Hall	1955	+2·28	−4·91	+2·63
	Hall	1956	−3·60	−5·20	+8·80
	Royalla "A"	1955	−2·80	−7·30	+10·10
	Royalla "B"	1956	−2·15	+0·30	+1·85
	Williamsdale	1956	−4·83	−1·81	+6·64
	15 small samples (total)		−15·89	+10·61	+5·28
			−26·95	−16·74	+43·69
Tid/Tid	Wombat	1956	−0·36	+2·95	−2·59
	Hall	1955	−1·14	−3·33	+4·47
	Hall	1956	−1·06	−0·52	+1·58
	Royalla "A"	1955	+0·10	−0·97	+0·87
	Royalla "B"	1956	−0·11	−3·12	+3·23
	Williamsdale	1956	−0·15	−0·05	+0·20
	15 small samples (total)		−0·26	−2·13	+2·39
			−2·98	−7·17	+10·15

large samples, for purposes of comparison. The consistency between these 15 samples and the other six is less than between the large samples, no doubt because of the considerable sampling errors in some of these small samples. It is fairly clear, however, that the overall pattern of the "interaction effect" is that shown diagrammatically in Figure 4, i.e. a negative interaction between Standard *EF* and Blundell *CD* chromosomes at the homozygous level (i.e. in *Bl/Bl*, *St/St* homozygotes) and

another negative interaction in those genotypes where Tidbinbilla chromosomes coexist in either the heterozygous or the homozygous condition with Standard *CD* chromosomes. A formal statistical analysis of the data for the six large samples by Dr. B. Griffing (see Appendix I) confirms the existence of a genetic interaction between the two heterotic systems.

In order to estimate the combined effects of the two heterotic mechanisms and the interaction between them, we have calculated the relative viabilities of the nine genotypes by the method of Haldane (1956), which includes a correction to

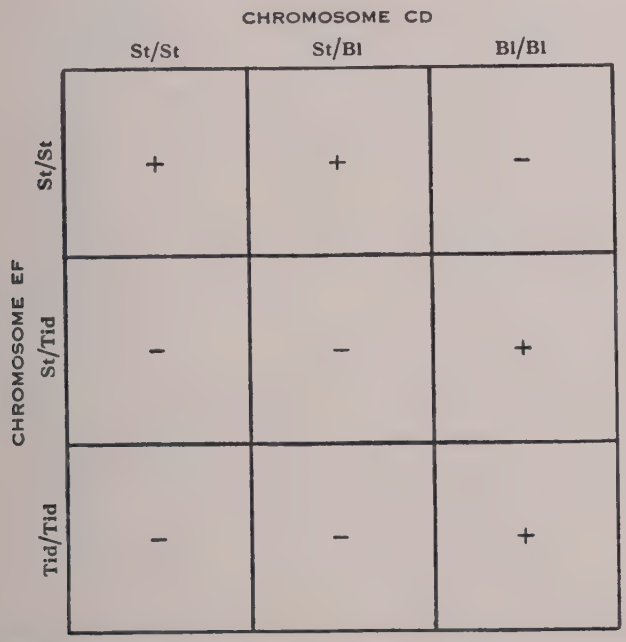


Fig. 4.—The characteristic pattern of positive and negative deviations from expectation due to the "interaction effect" (compare with Table 7). Almost exactly the same pattern is obtained when one calculates the relative viabilities by comparing the observed numbers with those expected on the basis of the Hardy-Weinberg ratio (Table 8).

remove bias. This calculation has been carried out separately for each of the six large samples, using the *St/Bl*, *St/St* genotype as an arbitrary standard (Table 8). Too much importance should not be attached to the values in those cells of the 3×3 table where the number of individuals observed was small (i.e. the three cells of the bottom row especially), since the sampling errors will obviously be large. Thus we do not believe that the *St/St*, *Tid/Tid* genotype is actually lethal at Hall and Wombat, although the fact that we did not find any individuals of this genotype at either locality does probably mean that such individuals are less viable at those places than in the Royalla–Williamsdale area. For the more common genotypes the relative viabilities as calculated in Table 8 are clearly meaningful. As was already suggested by Table 7, the negative interaction between Tidbinbilla *EF* and

Standard *CD* chromosomes reduces the viability of the *St/Bl*, *St/Tid* double heterozygotes below the viabilities of the single *St/Bl*, *St/St*, and *Bl/Bl*, *St/Tid* heterozygotes, which have the highest viability. In this way the two heterotic systems may be regarded as antagonistic or subtractive rather than additive.

TABLE 8

ESTIMATED RELATIVE VIABILITIES OF THE NINE GENOTYPES, CALCULATED FROM THE DEVIATIONS FROM EXPECTATION (ON THE BASIS OF THE HARDY-WEINBERG RATIOS) BY THE METHOD OF HALDANE (1956)*

St/Bl, *St/St* arbitrarily taken as 1·000 in each sample

Locality	Year of Collection	Chromosome *EF*	Chromosome *CD*		
			St/St	*St/Bl*	*Bl/Bl*
Hall	1955	*St/St*	0·871	1·000	0·904
Hall	1956		0·892	1·000	0·841
Royalla "A"	1955		0·949	1·000	0·864
Royalla "B"	1956		0·791	1·000	0·834
Wombat	1956		0·789	1·000	0·922
Williamsdale	1956		1·183	1·000	0·877
Mean viability			0·913	1·000	0·874
Hall	1955	*St/Tid*	1·055	0·969	1·015
Hall	1956		0·498	0·826	0·967
Royalla "A"	1955		0·895	1·001	1·114
Royalla "B"	1956		0·670	1·006	0·901
Wombat	1956		0·801	0·876	1·004
Williamsdale	1956		0·930	1·035	1·364
Mean viability			0·808	0·952	1·061
Hall	1955	*Tid/Tid*	0·000	0·255	1·186
Hall	1956		0·000	1·211	1·576
Royalla "A"	1955		0·680	0·650	0·726
Royalla "B"	1956		0·657	0·657	1·067
Wombat	1956		0·000	1·308	0·645
Williamsdale	1956		0·809	0·756	0·866
Mean viability			0·358	0·806	1·011

* Haldane's formula is as follows: Where we obtain a individuals of genotype A, and b of genotype B, instead of the expected numbers of a' and b', then $a'/b' \times b/(a+1)$ gives an unbiased estimate of the viability of B if the viability of A is 1.

We should not expect that the relative viabilities of the genotypes would be precisely the same in all localities and in all years, so that the fairly good agreement between the values for the six samples, in the case of those genotypes where the totals are fairly large, is interesting. The inconsistencies of Table 8 are probably only in part due to sampling error—to some extent, at any rate, they probably reflect

genuine differences in relative viabilities from place to place and perhaps also from year to year. In spite of this the means of the values for the six samples given in Table 8 probably give the best available estimate of the combined effect of the two interacting heterotic systems, not for any particular locality, but for the type of colony, common on the Southern Tableland of New South Wales, in which the frequency of Blundell exceeds 50 per cent. and that of Tidbinbilla is between 10 and 25 per cent. It is fairly obvious that the relative viabilities must diverge considerably from this pattern at a locality such as Michelago, where the chromosomal frequencies are very different from those found at Wombat, Hall, Royalla, and Williamsdale. In the majority of colonies where both polymorphisms are present one would expect that the *St/St, Tid/Tid* karyotype would be the rarest of the nine, and in fact only eleven individuals of this constitution were seen, out of over 8000 examined in the course of two years. However, at Michelago, Adelong, and Kyeamba the rarest genotype would be expected to be *Bl/Bl, Tid/Tid*. Possibly in the area occupied by these populations the interaction between the two heterotic systems is not present, or is of a different type to that which occurs in the populations of which we have large samples. Further south, at Beechworth, Vic., we even have a population which seems to be monomorphic for the Standard *CD* chromosome, but which possesses the *EF* polymorphism, a state of affairs quite unknown on the Southern Tableland and south-western slopes of New South Wales.

VI. Genetic Stability of the Populations in Space and Time

We had originally hoped to be able to make a careful comparison between the composition of the populations in 1955 and 1956, in order to determine if any significant changes had occurred from one year to the next. For various reasons it was only at Hall cemetery that a sufficiently large sample for an adequate comparison could be obtained in both years. It is not entirely clear whether any change did take place in the composition of the Hall population between these two years. If one compares the frequencies of the nine genotypes in 1955 and 1956 (Table 9) one obtains a χ^2 of 12·896, which, for 7 degrees of freedom, is not significant. The frequencies of the Standard and Blundell sequences of the *CD* chromosome are almost exactly the same in the two years. On the other hand, there was an apparent fall in the frequency of Tidbinbilla from 14·86 per cent. in 1955 to 12·00 per cent. in 1956, which is just significant on a χ^2 test ($P < 0{\cdot}05$). However, it would seem best not to lay too much stress on this; if the frequency of Tidbinbilla was not quite uniform over the entire area of 3–4 acres, minor differences in the extent to which the 1955 and 1956 samples were drawn from different sections of the cemetery could have been responsible for the apparent change in the frequency of Tidbinbilla. At Michelago cemetery, no significant change in the composition of the population took place between the two years. Meagre though these data are, they support the opinion previously expressed on general grounds that the populations are probably rather stable in composition from year to year (White 1956) so that very large samples would be needed to detect the slight changes which must undoubtedly occur.

From time to time, in the course of this work, we have suspected that there might be minor spatial differences in the composition of the populations, over an

area of several acres, even if the colony was continuous over the whole area. Microgeographic differences in population density, often not obviously correlated with any change in the vegetation, undoubtedly occur. The detection of microgeographic differences in the composition of animal populations is, however, notoriously difficult. In the case of the Wombat cemetery, which is an area of about 6 acres, three collections (July 5, July 14, and August 6) were mainly from a well-drained slope on the NW. side of the cemetery. Three other collections (April 27, July 20, and July 27)

TABLE 9

COMPOSITION OF THE HALL POPULATION IN 1955 AND 1956

Genotype	1955		1956		Totals
	Observed	Expected	Observed	Expected	
St/St, St/St	52	55·298	46	42·702	98
St/Bl, St/St	256	269·157	221	207·843	477
Bl/Bl, St/St	250	254·486	201	196·514	451
St/St, St/Tid	22	16·364	7	12·636	29
St/Bl, St/Tid	87	77·305	50	59·695	137
Bl/Bl, St/Tid	98	90·847	63	70·153	161
St/St, Tid/Tid	0	0	0	0	0
St/Bl, Tid/Tid	2	3·950	5	3·050	7
Bl/Bl, Tid/Tid	10	9·593	7	7·407	17
Totals*	777	777·000	600	600·000	1377
EF chromosome					
Standard	1323	1342·399	1056	1036·601	2379
Tidbinbilla	231	211·601	144	163·399	375
Totals†	1554	1554·000	1200	1200·000	2754

* $\chi^2_{(7)} = 12{\cdot}896$, $P > 0{\cdot}05$.
† $\chi^2 = 4{\cdot}725$, $P < 0{\cdot}05$.

were mainly from a low-lying area on the NE. and E. side of the cemetery. Comparing the frequencies of the nine genotypes on these two areas (Table 10) one obtains a χ^2 of 25·48 which, for 7 degrees of freedom, is highly significant. However, when one compares the frequencies of the *CD* and *EF* chromosome sequences on the two areas, there are no significant differences. The interesting thing here is that if we test for the interaction effect in the subsamples from the two areas we find that in the one from the well-drained slope the interaction effect is highly significant, even by a χ^2 test (Table 11). It accordingly appears as if the effect may be present in some habitats and not in others a few dozen yards away, and that perhaps the soil type or soil drainage has something to do with whether it is expressed or not. We shall give some reasons later for supposing that differences in viability, due to heterosis or genetic interaction, are probably manifested during the egg stage. If so, it

would not be surprising if there were very local differences in the relative viabilities of the various genotypes. It may be that the most important factor in this connection is the amount of moisture in the top centimetre of soil in midsummer, when the eggs are undergoing development. But it would require a special investigation to test any such possibility adequately.

TABLE 10

COMPOSITION OF WOMBAT CEMETERY POPULATION

Genotype	Well-drained Slope		Low-lying Area		Totals
	Observed	Expected	Observed	Expected	
St/St, St/St	5	6·524	9	7·476	14
St/Bl, St/St	100	91·802	97	105·198	197
Bl/Bl, St/St	237	237·194	272	271·806	509
St/St, St/Tid	0	2·330	5	2·670	5
St/Bl, St/Tid	17	28·426	44	32·574	61
Bl/Bl, St/Tid	103	90·870	92	104·130	195
St/St, Tid/Tid	0	0	0	0	0
St/Bl, Tid/Tid	0	3·728	8	4·272	8
Bl/Bl, Tid/Tid	4	5·126	7	5·874	11
Totals*	466	466·000	534	534.000	1000
CD chromosome					
Standard	127	141·664	177	162·336	304
Blundell	805	790·336	891	905·664	1696
Totals†	932	932·000	1068	1068·000	2000
EF chromosome					
Standard	804	792·666	897	908·334	1701
Tidbinbilla	128	139·334	171	159·666	299
Totals‡	932	932·000	1068	1068·000	2000

* $\chi^2_{(7)} = 25{\cdot}48$, $P < 0{\cdot}01$.
† $\chi^2_{(1)} = 3{\cdot}352$, $P > 0{\cdot}05$.
‡ $\chi^2_{(1)} = 2{\cdot}030$, $P > 0{\cdot}1$.

VII. DISCUSSION

M. scurra is an insect whose motility is very limited. Individuals live and die within a few metres of the point where they hatched from the egg. Prolificity is low (the maximum number of eggs obtained from one female, in captivity, was 21). There is never more than one generation a year. Thus the population dynamics of *M. scurra* resembles that of a land mollusc more than that of *Drosophila* spp., which have greater potential mobility and prolificity and many generations a year.

At the present time, many colonies of *M. scurra* consist of only a few dozen individuals in each generation. A colony of 1000 is "large". Removal of 777 males

from Hall cemetery in 1955 led to a situation in which no more could be found; in 1956 the colony appeared considerably smaller and after 600 males had been collected it was decided that there was no chance of finding another 100. It thus seems probable that this colony does not normally consist of more than 5000 individuals of both sexes, on an area of about $3\frac{1}{2}$ acres. The largest colony we have seen is almost certainly the one at Wombat cemetery, where 1000 males were collected without difficulty, although they were much harder to find towards the end of the collecting period. This cemetery may perhaps have contained at most 10,000 individuals of both sexes in 1956. On account of the low mobility of the species the genetically effective population size is undoubtedly smaller than the total number of individuals in the colony.

TABLE 11

THE "INTERACTION EFFECT" AT WOMBAT CEMETERY

Observed numbers of male individuals, with deviations from expectation, calculated from marginal totals

A. Total Collection

EF Chromosome	*CD* Chromosome			Totals
	St/St	*St/Bl*	*Bl/Bl*	
St/St	14(+0·320)	197(+5·480)	509(−5·800)	720
St/Tid	5(+0·041)	61(−8·426)	195(+8·385)	261
Tid/Tid	0(−0·361)	8(+2·946)	11(−2·585)	19
Totals*	19	266	715	1000

B. Samples from Well-drained Area on NW. Side

St/St	5(+1·330)	100(+14·133)	237(−15·463)	342
St/Tid	0(−1·287)	17(−13·129)	103(+14·416)	120
Tid/Tid	0(−0·043)	0(−1·004)	4(+1·047)	4
Totals†	5	117	344	466

* $\chi^2_{(4)} = 4{\cdot}202$ (n.s.).
† $\chi^2_{(4)} = 14{\cdot}527$, $P < 0{\cdot}01$.

In colonies which persist precariously in such localities as the cemeteries at Windellama and Bong Bong (probably less than 50 individuals per generation on small areas) a significant amount of inbreeding must occur. And even in a population the size of the Wombat one it may well be that the frequency of consanguineous matings (sib-sib, first cousin, second cousin, etc.) is far higher than would be the case if the whole population were randomized in each generation. If so, there would be a significant departure from panmixia, and it would not be appropriate to calculate deviations from the expectation on the basis of the Hardy-Weinberg equilibrium,

which is the basis for the Levene calculations in Tables 5 and 6, and for the estimation of relative viabilities in Table 8.

However, an excess of consanguineous matings will produce an excess of homozygotes, not a deficiency. Thus it is possible that the heterotic effects on viability, discussed in Section IV, may be greater than we have calculated them to be, but they can hardly be less. And as far as the interaction effect is concerned, it is not of a type that could be due to inbreeding in small isolates, which would be expected to lead to excesses of all homozygous genotypes and deficiencies of heterozygous ones.

Such colonies as those at Windellama and Bong Bong must have been isolated for many generations. The first of these cemeteries dates from the late 1850's and the second from about 1840. It is, of course, probable that in the early days of settlement by white men the colonies inside the cemeteries were continuous with much larger populations outside, before the destruction of the native *Themeda* grassland by sheep grazing. We cannot know the date at which the populations of *M. scurra* outside the cemetery fences became extinct, but it may have been well before 1900 in some instances.

We believe that the differential mortality of the various genotypes of *M. scurra* must occur quite early in the life cycle, and certainly no later than the early nymphal instars. There is no suggestion in our data that the deficiency of homozygotes increases over the period April–October, as it would do if the differential mortality occurred in the adults. And we have the most definite impression that the populations do not undergo any considerable decline in numbers during this part of the life cycle, i.e. that an individual which is alive in April has a 90 per cent or better chance of remaining alive until October. If this is so, the total mortality during the April–October period is not sufficient to account for the differential effects calculated, and the selective mortality must occur at an earlier stage.

It is well known that in many grasshopper species the largest mortality is in the egg stage (Richards and Waloff 1954). Thus we are predisposed to favour the hypothesis that the differential survival of cytological homozygotes and heterozygotes in *M. scurra* occurs among the eggs. This might lead to differences in the pattern of selection over very short distances such as a few yards, depending on soil type, extent of vegetational cover, etc.

The wide distribution of the main chromosome rearrangements of *M. scurra* (i.e. Blundell and Tidbinbilla and the corresponding Standard sequences) argues in favour of their great antiquity. Hundreds of thousands of generations (i.e. years) must have been required to produce the present geographic distribution of these inversions. Their persistence over such a long period of time, in so many colonies of the species, argues in favour of balanced polymorphisms based on true heterosis. At least we would imagine that polymorphisms which depended for their preservation solely on adaptation to different environments in time or space (without heterosis) would be unlikely to have persisted through all the vicissitudes of such a long period of evolution.

It is, however, an open question whether a particular sequence such as Blundell has essentially the same genetic properties (at least as far as the heterotic mechanism is concerned) in all localities (including those where it is very common and those

where it is rare). The very great differences in the relative abundance of the *CD* chromosome sequences at such geographically close localities as Carcoar and Lyndhurst or Williamsdale and Michelago may reflect environmental differences between these localities, particularly if it is the physical properties of the soil (or perhaps its microflora) which are significant in this respect rather than more easily measured meteorological factors such as rainfall, temperature, etc. However, it is also probable that, for example, Blundell chromosomes at Carcoar may have genetic properties similar to those of Standard chromosomes at Lyndhurst and vice versa.

Since the interaction effect seems to occur over a large geographic area and is apparently present in both the 15- and 17-chromosome races of the species, it is presumably almost or quite as ancient as the actual heterotic mechanisms. If the *CD* and *EF* heterotic mechanisms both operate on the same physiological system or chain of biosynthetic processes, the interaction between them may be in some respects analogous to such interactions as those between the thalassemia and sickling genes in man (Allison 1955; Neel 1956).

Since the interaction between the Blundell and Tidbinbilla chromosomes is a favourable one, it appears at first sight as if a centric fusion between these two chromosomes should possess considerable selective advantage. No such fused *Bl–Tid* chromosome has been found in any of the populations studied, but in view of the rather large number of chromosomal rearrangements which are known to have arisen in *M. scurra* it seems likely that such fusions must have occurred from time to time in the history of the species. Although a rigorous mathematical proof would be difficult, it is probable that fusions of this type have been selected against (at least in populations of the Wombat–Hall–Royalla type) because of the rather large proportion of *Bl–Tid*/*St*,*St* double heterozygotes which would be produced (such double heterozygotes would have a lower viability than the *St*/*Bl*, *St*/*St*, and *Bl*/*Bl*, *St*/*Tid* single heterozygotes).

The genetic interaction between the two heterotic systems is clearly the reason for the observed correlation between the frequencies of the Blundell and Tidbinbilla sequences, and hence for the negative correlation between Tidbinbilla and the Standard *CD*, throughout the distribution area of the species. Presumably, those colonies which lack Tidbinbilla altogether are mostly ones in which, at some time in the past, the frequency of the Standard *CD* rose too high to permit the maintenance of Tidbinbilla in the population. At Beechworth, Vic., we do seem to have a population homozygous for the Standard *CD* which nevertheless contains the Tidbinbilla sequence in low frequency, but it is hardly probable that these sequences have the same genetic properties in Victoria as they do further north.

The picture of balanced chromosomal polymorphism which emerges from the present work is a rather complex one, since it seems to involve equilibria, not only between members of the same pair of chromosomes, but also between members of two different pairs. That genotypes are complexly organized systems has been apparent for some time—but there have been few studies of just how these organized genetic interactions operate in natural populations. We believe that interactions between adaptive polymorphisms may be expected wherever two or more such systems are concerned with the same physiological process or may be regarded as

adaptive responses to the same ecological challenge. Thus in the mollusc *Purpura lapillus* (Staiger 1954) a race with 18 acrocentric chromosomes in the haploid set inhabits sheltered habitats. No less than five different chromosomal fusions all seem to help in adapting the species to intertidal habitats exposed to strong wave action. Polymorphic colonies only occur in ecologically intermediate localities. Just what biochemical or physiological mechanisms underlie the five presumed heterotic systems in this instance cannot be guessed at. But it is the kind of case in which we would expect to find evidence of genetic interaction between them, since their relationship to the ecological environment suggests that their genetic properties are all similar, or are at any rate adaptive to the same type of habitat. Kimura (1956) has shown mathematically that genetic interaction of the type which we believe to occur in the populations of *M. scurra* cannot lead to a stable equilibrium in the absence of heterosis. But where several non-linked chromosomal polymorphisms are present in a population, each independently giving heterotic effects, there should frequently be complex interactions between them which will modify the equilibria in various ways.

VIII. Acknowledgments

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Appendix I

Statistical analysis of the data for the six large samples

The objective in the following analyses is to examine the genetic mechanisms which may account for the stable polymorphic equilibrium which exists simultaneously in the *CD* and *EF* chromosome combinations in six populations.

Table 12

Representation of the two-way classification of the data involving the *CD* and *EF* chromosomal combinations

Chromosome *EF*	Chromosome *CD*			Row Total	Theoretical Proportion
	St/St	*St/Bl*	*Bl/Bl*		
St/St	n_{11}	n_{12}	n_{13}	$n_{1\cdot}$	p_1
St/Tid	n_{21}	n_{22}	n_{23}	$n_{2\cdot}$	p_2
Tid/Tid	n_{31}	n_{32}	n_{33}	$n_{3\cdot}$	p_3
Column total	$n_{\cdot 1}$	$n_{\cdot 2}$	$n_{\cdot 3}$	n	
Theoretical proportion	q_1	q_2	q_3		1

This equilibrium phenomenon can conceivably be maintained in at least two ways. It can be maintained by overdominance (superiority of the heterozygote over both homozygotes) operating in each chromosome pair independently, or by overdominance operating in each chromosome pair together with some form of interaction between chromosome pairs. According to Kimura (1956) interaction between chromosome pairs without overdominance cannot maintain a balanced polymorphism.

The statistical objective then is to detect and test for interactions between homologous chromosomes and between chromosome pairs.

In these analyses we must assume that survival to a given age is the primary cause, or, as an element in a system of causes, it is highly correlated with the entire causal system which maintains the polymorphic condition. Since the data are obtained from males only, we must also assume that the viability constants associated with the various chromosomal combinations are the same for both sexes.

The data take the form of a 3×3 matrix as illustrated in Table 12. The columns are associated with the different *CD* (Blundell–Standard) chromosomal combinations

(i.e. *St/St*, *St/Bl*, *Bl/Bl*) and the rows are associated with the *EF* (Tidbinbilla–Standard) combinations (i.e. *St/St*, *St/Tid*, and *Tid/Tid*). n_{ij} denotes the observed number of individuals having the ith *EF* and the jth *CD* chromosomal types; $n_{i.}$ ($i = 1, 2,$ and 3) are the row marginal totals and $n_{.j}$ ($j = 1, 2,$ and 3) are the column marginal totals and n is the total. The expected row proportions are denoted by p_i and the column proportions by q_j..

This pattern of data allows for a goodness-of-fit χ^2 with a maximum of eight degrees of freedom. This can be partitioned into orthogonal components each associated with a single degree of freedom (Table 13) in a manner analogous to the partitioning of a sum of squares in an analysis of variance of a 3^2 factorial design. The χ^2 associated with the main effects of each classification can be partitioned into linear and quadratic components each with one degree of freedom. The interaction χ^2 can be partitioned exactly into four pairwise orthogonal contrasts based on the linear and quadratic functions of each classification. Thus, it can be shown that the following algebraic identity exists:

$$\chi^2 \equiv \sum_i^3 \sum_j^3 \frac{(n_{ij} - np_iq_j)^2}{np_iq_j} \equiv \sum_k^8 Y_k^2,$$

where χ^2 is the total χ^2 associated with the 3×3 table and Y_k ($k = 1, \ldots 8$) are the eight orthogonal contrasts each of which is divided by the square root of its appropriate divisor.

In any given test the actual number of degrees of freedom associated with the total χ^2 for the 3×3 table is determined by the number of parameters estimated from the data. It is conceivable that certain hypotheses to be tested give theoretical marginal frequencies *a priori*. In such cases all eight degrees of freedom are available and each contrast yields an independent χ_1^2. However, if the hypothesis requires that a single parameter be estimated from the row marginal totals and one from the column marginal totals, then a single degree of freedom is lost from each of the main effects χ^2's, and for each classification the linear and quadratic components are pooled to give a combined χ^2 with one degree of freedom. Finally, if the hypothesis requires that the observed marginal frequencies be used to estimate the expected marginal frequencies, then all "main effects" contrasts equal zero and the total χ^2 collapses into the interaction χ^2 with four degrees of freedom.

To give a genetic interpretation to the partitions of the main effects we shall consider the characterization of heterosis in terms of the linear and quadratic contrasts. The following example outlines the interpretation which can be attached to these contrasts. Let us consider a population which in the egg stage has a random mating frequency for the distribution of the karyotypes of a single chromosome pair. Heterosis in the adult population is manifested by the differential mortality of the various classes of karyotypes such that the homozygotes are less viable than the heterozygotes. However, it may be that one homozygous class is much less viable than the other. Therefore a complete description of the heterosis phenomenon must take into consideration not only the relative advantage of the heterozygote over the homozygotes (measured by the quadratic contrast) but also the disproportionate mortality of the two classes of homozygotes (measured by the linear contrast).

TABLE 13

PARTITIONING OF THE TOTAL χ^2 INTO ORTHOGONAL COMPONENTS

Main effects *EF*	$L_T = \dfrac{[p_3(n_{1\cdot}) - p_1(n_{3\cdot})]^2}{np_1p_3(p_1+p_3)}$
	$Q_T = \dfrac{[(p_1+p_3)(n_{2\cdot}) - p_2(n_{1\cdot}+n_{3\cdot})]^2}{np_2(p_1+p_3)}$
Main effects *CD*	$L_B = \dfrac{[q_3(n_{\cdot 1}) - q_1(n_{\cdot 3})]^2}{nq_1q_3(q_1+q_3)}$
	$Q_B = \dfrac{[(q_1+q_3)(n_{\cdot 2}) - q_2(n_{\cdot 1}+n_{\cdot 3})]^2}{nq_2(q_1+q_3)}$
Interaction	$L_T \times L_B = \dfrac{[p_3q_3(n_{11}) - p_3q_1(n_{13}) - p_1q_3(n_{31}) + p_1q_1(n_{33})]^2}{np_1p_3q_1q_3(p_1+p_3)(q_1+q_3)}$
	$L_T \times Q_B = \dfrac{[p_3q_2(n_{11}+n_{13}) - p_3(q_1+q_3)(n_{12}) - p_1q_2(n_{31}+n_{33}) + p_1(q_1+q_3)(n_{32})]^2}{np_1p_3q_2(p_1+p_3)(q_1+q_3)}$
	$Q_T \times L_B = \dfrac{[p_2q_3(n_{11}+n_{31}) - p_2q_1(n_{13}+n_{31}) - (p_1+p_3)q_3(n_{21}) + (p_1+p_3)q_1(n_{23})]^2}{np_2q_1q_3(p_1+p_3)(q_1+q_3)}$
	$Q_T \times Q_B = \dfrac{[p_2q_2(n_{11}+n_{13}+n_{31}+n_{33}) - p_2(q_1+q_3)(n_{12}+n_{32}) - (p_1+p_3)q_2(n_{21}+n_{23}) + (p_1+p_3)(q_1+q_3)(n_{22})]^2}{np_2q_2(p_1+p_3)(q_1+q_3)}$

The genetic interpretation of the linear and quadratic contrasts can be stated exactly for the situation in which a stable equilibrium is maintained by the superiority of the heterozygotes over both homozygotes. Consider the chromosomal combinations A_1A_1, A_1A_2, and A_2A_2, having the relative viabilities $1-v_1$, $1-v_2$, and $1-v_3$ respectively. It is well known that in a large random mating population a stable equilibrium is possible only when $v_1 > v_2$ and $v_3 > v_2$. Under these conditions the expected equilibrium frequencies (after selection has taken place) are:

For A_1A_1:

$$m_1 = \frac{(v_3-v_2)^2(1-v_1)}{ab},$$

for A_1A_2:

$$m_2 = \frac{2(v_1-v_2)(v_3-v_2)(1-v_2)}{ab},$$

and for A_2A_2:

$$m_3 = \frac{(v_1-v_2)^2(1-v_3)}{ab},$$

where

$$a = v_1-2v_2+v_3,$$

and

$$b = v_1-2v_2+v_3+v_2^2-v_1v_3.$$

The expected chromosomal frequencies are:

For A_1:

$$f_1 = \frac{v_3-v_2}{a},$$

and for A_2:

$$f_2 = \frac{v_1-v_2}{a}.$$

The expected karyotypic frequencies (based on f_1 and f_2), assuming random mating and no selection are:

For A_1A_1:

$$m_1' = \frac{(v_3-v_2)^2}{a^2},$$

for A_1A_2:

$$m_2' = \frac{2(v_1-v_2)(v_3-v_2)}{a^2},$$

and for A_2A_2:

$$m_3' = \frac{(v_1-v_2)^2}{a^2}.$$

The χ^2 involving the deviations (m_i-m_i') can be shown to be equal to

$$\chi^2 = \sum_i \frac{n(m_i-m_i')^2}{m_i'} = K[(v_3-v_1)^2+2(v_1-v_2)(v_3-v_2)],$$

where

$$K = \frac{n(v_1-v_2)^2(v_3-v_2)^2}{b^2[(v_1-v_2)^2+(v_3-v_2)^2]}.$$

If we compute

$$L = \frac{n(m_3'm_1 - m_1'm_3)^2}{m_1'm_3'(m_1' + m_3')} = K(v_3 - v_1)^2,$$

and

$$Q = \frac{n[(m_1' + m_3')m_2 - m_2'(m_1 + m_3)]^2}{m_2'(m_1' + m_3')} = 2K(v_1 - v_2)(v_3 - v_2),$$

we note that χ^2 is partitionable into two parts (i.e. $\chi^2 = L + Q$), that the linear component involves a contrast of viability constants of the homozygotes (viz. $L = K(v_3 - v_1)$), and that the quadratic component involves the differences in viability constants of the homozygotes and the heterozygote (viz. $Q = 2K(v_1 - v_2)(v_3 - v_2)$).

Because the selective forces are small and sample sizes are necessarily restricted, we wish to use tests which are as sensitive as possible. We shall, therefore, not use the χ^2 tests directly, but shall convert the components of χ^2 into normal deviates (i.e. the transformed variates are considered to be normally distributed with zero mean and variance equal to one) and use appropriate tests on these. The increase in sensitivity in our case is due primarily to the fact that the signs associated with the normal deviates are important in respect to the hypotheses being tested. Thus instead of using $L = (N_L)^2 D_L$, where N_L is the numerator before squaring (i.e. the contrast) and D_L is the denominator, we shall use $Y_L = N_L (D_L)^{\frac{1}{2}}$. Instead of using $Q = (N_Q)^2 D_Q$, we shall use $Y_Q = N_Q (D_Q)^{\frac{1}{2}}$. Under conditions of a stable equilibrium

$$Y_L = K^{\frac{1}{2}}(v_3 - v_1),$$

and the sign of Y_L is determined by $(v_3 - v_1)$. Similarly,

$$Y_Q = K^{\frac{1}{2}}[2(v_1 - v_2)(v_3 - v_2)]^{\frac{1}{2}},$$

and, if the conditions of a stable equilibrium hold, Y_Q is necessarily positive.

Examination of Data

Data from the six largest samples only will be considered. These data are given in Table 1.

We shall first test the hypothesis that overdominance exists in each chromosome pair. This involves only the first four contrasts associated with the main effects. Then, we shall test for interaction between the two chromosome pairs, assuming that interaction within each chromosome pair does exist. The interaction contrasts are involved in this test.

The normal deviates associated with the main effects of each chromosome pair are given in Table 14. The subscript in front of the normal deviate designates the chromosome pair. For example ${}_8Y_L$ is the normal deviate associated with the linear contrast of the CD pair and ${}_7Y_L$ is the similar deviate for the EF pair.

For each contrast homogeneity of the data from the different localities may be determined by comparing the estimated variance within the contrast with the value one. For each of the four classes of normal deviates we find that the data are homogeneous.

Another method of detecting heterogeneity of the data is to consider an analysis of variance involving data of ${}_TY_Q$ and ${}_BY_Q$. (Data for ${}_TY_L$ and ${}_BY_L$ cannot be included because the sign associated with these normal deviates is determined by the somewhat arbitrary classification as to which chromosome of each pair is considered "standard".) The analysis of variance is given in Table 15. Since the data are considered normal deviates, the sum of squares may be considered as χ^2. None is significantly large. Therefore, we assume (i) that there are no real differences between locations for these two contrasts; (ii) mean values for the two contrasts are not different for these locations; and (iii) there is no location $\times$ contrast interaction.

TABLE 14

NORMAL DEVIATES ASSOCIATED WITH THE MAIN EFFECTS OF EACH CHROMOSOME PAIR FOR SIX LOCATIONS

Location	*CD* Chromosome		*EF* Chromosome	
	${}_BY_L$	${}_BY_Q$	${}_TY_L$	${}_TY_Q$
Wombat	−0·813,419	0·593,389	0·676,391	0·486,578
Williamsdale	0·129,861	−0·829,713	0·852,435	1·072,330
Hall (1955)	−0·337,812	0·608,306	1·191,410	0·852,981
Hall (1956)	−0·708,847	1·285,255	−1·111,552	−0·672,145
Royalla "A"	−0·267,267	0·501,139	1·530,455	1·777,644
Royalla "B"	−0·958,514	1·917,029	0·328,575	0·315,206
Mean ($\bar{x}$)	−0·492,666	0·679,234	0·577,952	0·638,765
Variance	0·165,791	0·844,088	0·857,047	0·675,450
Probabilities	$P \cong 0{\cdot}1131$	$P \cong 0{\cdot}0485$	$P \cong 0{\cdot}0778$	$P \cong 0{\cdot}0594$

TABLE 15

ANALYSIS OF VARIANCE INVOLVING THE QUADRATIC NORMAL DEVIATES ${}_BY_Q$ AND ${}_TY_Q$ FOR SIX LOCATIONS

Source	D.F.	Sum of Squares	
Between locations	5	1·744,719	n.s.
Between contrasts	1	0·004,913	n.s.
Locations × contrasts	5	5·852,969	n.s.

Since, for any given contrast, we assume that the data are homogeneous, we may pool the data and test for deviation of the contrast from zero. The test involves $t = \bar{x}/s_{\bar{x}}$, where $s_{\bar{x}} = (\frac{1}{6})^{\frac{1}{2}}$. The statistic t is treated as a normal deviate. The probabilities given in Table 14 are based on a one-tailed test.

The hypothesis considered at this stage, which is the alternative to the random mating and no-selection hypothesis, is that for each chromosome pair the populations

are in a stable equilibrium because of the relative superiority of the heterozygote. The *t*-tests support this hypothesis of overdominance of each chromosome pair, and it appears that both linear and quadratic aspects of heterosis are evident.

Assuming that overdominance exists in each chromosome pair, we shall now test for interaction between chromosome pairs. In this test the null hypothesis assumes that the stable equilibrium condition is brought about *independently* in each chromosome system by the superiority of the heterozygote over both homozygotes. The test for interaction involves the χ^2_4 obtained from the 3×3 contingency table in which the cell probabilities are estimated as the product of the observed marginal frequencies. This interaction χ^2_4 is partitionable into four orthogonal components based on linear and quadratic contrasts as given in Table 13.

As before, instead of taking the actual χ^2 components, we shall use the normal deviates derived from these components. Thus we shall use

$$Y_{L\times L} = N_{L\times L}/(D_{L\times L})^{\frac{1}{2}},$$
$$Y_{L\times Q} = N_{L\times Q}/(D_{L\times Q})^{\frac{1}{2}},$$
$$Y_{Q\times L} = N_{Q\times L}/(D_{Q\times L})^{\frac{1}{2}},$$
$$Y_{Q\times Q} = N_{Q\times Q}/(D_{Q\times Q})^{\frac{1}{2}},$$

where N and D are defined as before. These normal deviates are given in Table 16 for each contrast together with the contrast means, variances, and the probability levels associated with tests on the contrast means.

TABLE 16

NORMAL DEVIATES ASSOCIATED WITH THE INTERACTION OF THE TWO CHROMOSOME PAIRS FOR SIX LOCATIONS

Location	$Y_{L\times L}$	$Y_{L\times Q}$	$Y_{Q\times L}$	$Y_{Q\times Q}$
Wombat	0·494,240	1·435,661	0·093,740	−1·373,062
Williamsdale	0·485,016	−0·109,983	1·828,716	−0·425,709
Hall (1955)	1·747,379	−2·015,091	−0·417,233	−0·802,134
Hall (1956)	1·351,231	−0·381,281	1·671,126	−1·064,859
Royalla "A"	0·240,805	−0·568,605	1·112,362	−1·135,020
Royalla "B"	0·610,980	−1·331,966	0·662,959	0·052,122
Mean ($\bar{x}$)	0·821,608	−0·495,211	0·825,278	−0·791,444
Variance	0·347,942	1·381,045	0·782,018	0·275,312
Probabilities				
(one-tailed test)	$P \cong 0{\cdot}0222$	$P \cong 0{\cdot}1131$	$P \cong 0{\cdot}0217$	$P \cong 0{\cdot}0262$
(two-tailed test)	$P \cong 0{\cdot}0444$	$P \cong 0{\cdot}2262$	$P \cong 0{\cdot}0434$	$P \cong 0{\cdot}0524$

Since none of the variances between locations within contrasts is significantly greater than one, we cannot assume that the manifestation of chromosomal interaction is different in these locations. Therefore, for each contrast the data from the various locations may be pooled and the overall contrast means may be tested for deviations from zero. The statistic $t = \bar{x}/s_{\bar{x}}$ where $s_{\bar{x}} = (\frac{1}{6})^{\frac{1}{2}}$ is used as a normal deviate. Probabilities are given in Table 16 for both one- and two-tailed tests. Since

we are not testing a specific interaction hypothesis, we cannot specify the sign associated with the normal deviates of each contrast and therefore the two-tailed test is perhaps more appropriate.

From these tests it appears that interaction exists between these chromosome pairs and is manifested in at least three of the four interaction contrasts.

The inference from these analyses (when the assumptions we have made are taken into consideration) is that in these six populations the stable polymorphic equilibrium condition involving the two chromosome systems is due to both within and between chromosomal interactions.

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CYTOGENETICS OF THE GRASSHOPPER *MORABA SCURRA*

III. DISTRIBUTION OF THE 15- AND 17-CHROMOSOME RACES

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Summary

The 15- and 17-chromosome races of the wingless grasshopper *Moraba scurra* Rehn occupy areas which meet along a line at least 150 miles long, approximately separating the Southern Tableland of New South Wales from the south-western slopes. Within each area the species is at present confined to small colonies which only occur where grazing by sheep has been light or absent; but the populations of both races must have been far larger and more continuous a century ago.

In the area between Yass and Rugby numerous small colonies of the 15- and 17-chromosome races have been found, only a few miles apart, and in one instance separated by approximately 900 yd. In spite of this, only a single interracial hybrid (i.e. a 16-chromosome male) was encountered and no colony was found containing representatives of both races.

It is concluded that selection against chromosome-number heterozygotes (i.e. "negative heterosis") must be relatively strong. The distribution of the species as a whole in this area a hundred years ago must have resembled a jigsaw puzzle with perhaps a third of the pieces missing. Across such a pattern a zone possibly only a few hundred yards wide (and at most one or two miles wide) would have extended, within which overlap and interracial hybridization could have occurred. The origin of the 17-chromosome race is discussed in the light of these findings and interpretations.

I. INTRODUCTION

In earlier papers we have dealt with the mechanisms of adaptive chromosomal polymorphism in *Moraba scurra* Rehn (Orthoptera: Eumastacidae) (White 1956, 1957*b*). The cytology of hybrids between the 15- and 17-chromosome races has also been described (White 1957*a*). This work has shown (1) that the two races are truly members of a single species; (2) that no major genetic barriers to hybridization between them exist (minor mating preferences or differences in viability of hybrid offspring would not have been detected by the techniques used); and (3) that the difference in chromosome number results from a "dissociation" of a metacentric "*AB*" chromosome into two acrocentrics, "*A*" and "*B*", which has become fixed in the homozygous condition in the 17- chromosome race. This dissociation was a special type of translocation, probably involving a *CD* chromosome carrying the Blundell rearrangement.

Following on these studies it seemed desirable to survey intensively the zone separating the known distribution areas of the two races (see White 1956, Fig. 1) in order to determine as far as possible whether any overlap between the races exists, i.e. whether there is a zone within which mixed colonies occur. Such mixed colonies

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would be expected to contain individuals homozygous for the "fused *AB*" together with those homozygous for "broken *AB*" and the heterozygotes, with an *AB* chromosome, an *A*, and a *B*. The heterozygotes might be present in the frequency to be expected on the Hardy-Weinberg ratio, or in a greater or lesser frequency.

This survey was carried out by the junior author in the spring of 1956, as far as the area between Yass and Rugby, N.S.W., is concerned. Unfortunately, time did not permit the survey to be continued south-westward from Yass in the direction of Gundagai and Holbrook, although collecting by Dr. K. H. L. Key has shown that the 15-chromosome race occurs at Tumut, Batlow, and near Woomargama (see White 1957*b*, Fig. 1).

The task of carrying out this survey and interpreting the results was greatly complicated by the fact that, although *M. scurra* was undoubtedly a widespread species with a semi-continuous distribution a hundred years ago, it only survives today in isolated colonies where grazing by sheep has been absent or of low intensity. In general, the presence of the grass *Themeda australis* (R.Br.) Stapf may be taken as an indicator of areas favorable for *M. scurra*. Although the grasshopper feeds to a considerable extent on *Helichrysum apiculatum* (Labill.) DC. and other Compositae and apparently does not eat *Themeda*, overgrazed areas where *Helichrysum* is abundant but *Themeda* is absent do not support populations of the grasshopper. The evidence thus suggests that *Themeda* (or other perennial grasses, which may sometimes replace it) plays an essential role by providing the insect with "shelter" at some stage in its life cycle (whether "shelter" is from predators, from desiccation, or whether the grass clumps act in some other way is not known).

II. Observations

Previous work had shown that the area within which "mixed" colonies could be expected to exist was quite narrow. In 1955 we had found the 15-chromosome race at Yass cemetery and in a paddock about 1 mile south of Pudman Creek post-office and the 17-chromosome race at Rye Park, at a point about 5 miles north of Tangmangaroo and a couple of hundred yards north of Bowning. These localities define a "corridor" about 8 miles wide.

Figure 1 shows a map of the area on which are indicated all the localities at which *M. scurra* has now been collected. It will be seen that the corridor between the known distribution areas of the two races has now been reduced considerably—in two places to less than 5 miles wide and in one area (along Thieves' Creek, about 9 miles N. of Yass) to 900 yd.

No mixed colony, in the strict sense, has been found in the course of this work, i.e. one containing 15-, 16-, and 17-chromosome males with the corresponding 16-, 17-, and 18-chromosome females. Only at Thieves' Creek "A" was a single natural heterozygote (a 16-chromosome male) encountered together with eight males and four females of the 15-chromosome race. This small colony, which appeared to be existing somewhat precariously at a locality not very favorable for the species, is situated about 900 yd to the east of Thieves' Creek "B" where a total of 11 males belonging to the 17-chromosome race were collected among scattered clumps of *Themeda*. Most of the land between these two sites had been heavily grazed for many

years and carried no *Themeda*. The whole area is ecotonal between dry sclerophyll forest and savannah woodland, the original dominants being *Eucalyptus macrorrhyncha*

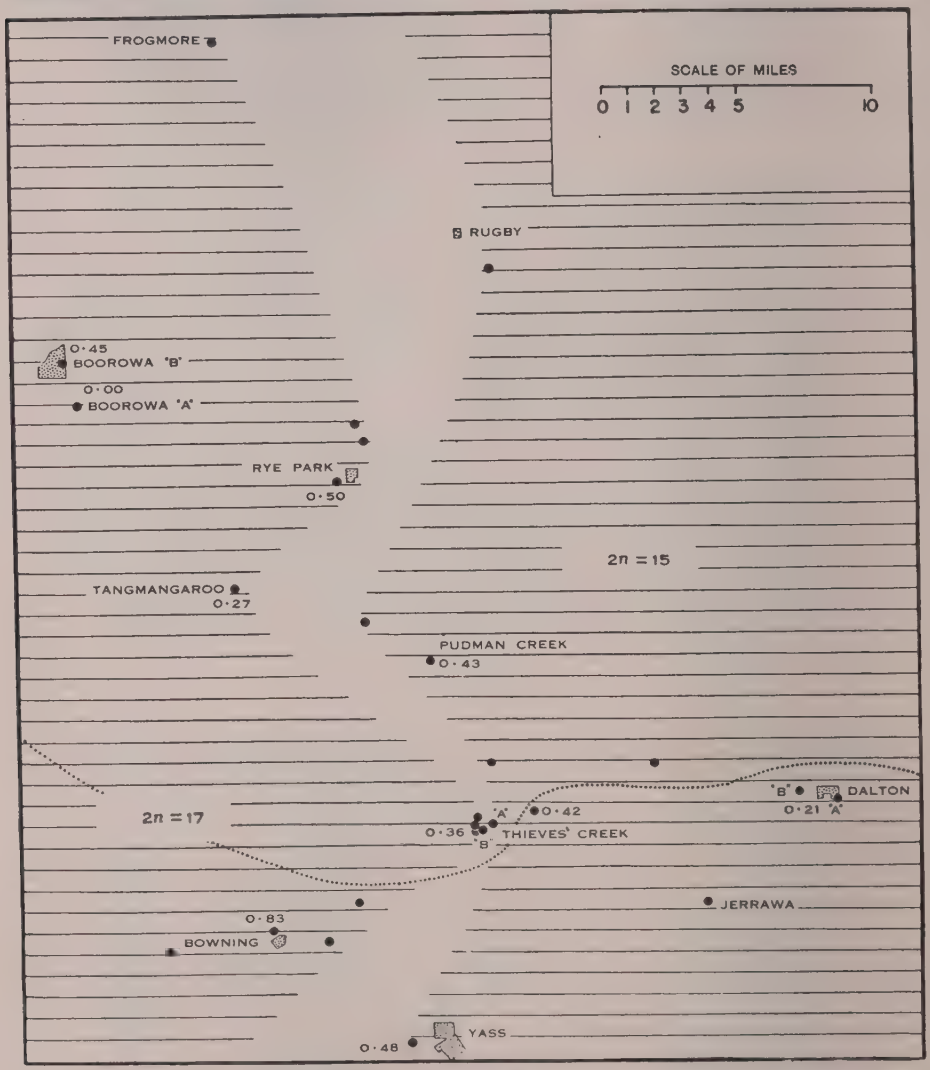

Fig. 1.—Map of a small part of central New South Wales showing the colonies of *M. scurra* discovered in 1955 and 1956. Those at Frogmore, Boorowa "A", Dalton "A", and Yass are cemetery populations, while that 2 miles ESE. of Bowning is on a railway exclosure. The figures alongside some of the localities represent the frequencies of the Blundell rearrangement. The Tidbinbilla rearrangement is present in the populations below the dotted line, but absent above that line. The blank space between the cross-hatched areas represents the "corridor" within which a zone of overlap could exist.

F. Muell., *E. stuartiana* F. Muell., *E. blakelyi* Maid., and (at site "A", but not at "B") *E. dives* Schau. and *E. rossii* Baker & Smith; however, it has been very largely cleared in

the course of development for grazing. Dr. Key pointed out to us that the forms of the crowns of the remaining mature trees confirm that the area was originally ecotonal and not pure dry sclerophyll forest. The small colonies of *M. scurra* were found only where *Themeda* persisted. It is clear from the whole ecology of the area that the *Themeda* must originally have been continuous or practically so between sites "A" and "B", whereas in 1956 there was a gap about 700 yd wide in which no *Themeda* was growing. Thus there can be no reasonable doubt that before the introduction of sheep the two races of *M. scurra* were in actual contact in this area.

Site "B" is near the eastern end of a large paddock at the western end of which (about 390 yd west of site "B") we found another colony of the 17-chromosome race (65 males analysed). We have called this site "9 miles north of Yass". The paddock was used for cattle at the time of our visits to it, and it is probable that the persistence of *Themeda* (and hence *M. scurra*) is due to the fact that it has been used for cattle rather than sheep. The next paddock to the eastward, in which site "A" is situated, has clearly been used for sheep and contains very little *Themeda* except at site "A" itself. This is located on a steep rocky slope above the small stream of Thieves' Creek, where the physiography has presumably lightened the grazing pressure.

These localities were visited on August 31, October 22, and October 29, 1956. The latter two dates are towards the end of the natural life cycle of the species, when the size of the populations would be expected to have declined somewhat. All males seen on each of these occasions were collected and it seems unlikely that the colonies at sites "A" and "B" could have consisted of more than 50 individuals each a few months earlier in the year.

It is entirely possible that, since the restriction of the general population of *M. scurra* in the Thieves' Creek area to a few isolated colonies, the variability at sites "A" and "B" may have diminished as a result of genetic drift. But drift has not led to the disappearance of the heterotic polymorphism associated with the Blundell and Standard sequences of the *CD* chromosome, either in the Thieves' Creek populations or in the equally small colonies in the cemeteries at Windellama and Bong Bong, although it may have done so at Boorowa cemetery (White 1956). The colony at Thieves' Creek "A" has clearly received some immigration of individuals of the 17-chromosome race in the past. The colony at site "B", on the other hand, showed no evidence of racial mixture, and if it ever did so in the past all fused *AB* chromosomes seem to have been lost. But the existence of a relatively large racially pure colony at the other end of the same paddock ("9 miles north of Yass") argues against the colony at site "B" having ever been racially heterogeneous, and the whole of the evidence presented in Figure 1 strongly suggests that the zone of overlap in the Thieves' Creek area was not much more than a thousand yards wide and may have been narrower. In the Yass–Bowning region and in the triangle between Rye Park, Tangmangaroo, and Pudman Creek the overlap zone could not have been wider than 5 miles and was probably no wider than at Thieves' Creek. Except for a few forested ridges, the whole of this area was probably suitable for *M. scurra* a century ago and was most likely occupied by a relatively dense and quasi-continuous population of the species.

In Table 1 we give the frequencies of the Blundell and Tidbinbilla rearrangements of the *CD* and *EF* chromosomes in the colonies near the interracial boundary (the data on which these are based are given by White 1957*b*). The following points seem worth mentioning: (1) the Tidbinbilla rearrangement is confined to the southern

TABLE 1

FREQUENCIES OF THE BLUNDELL AND TIDBINBILLA REARRANGEMENTS IN POPULATIONS NEAR THE INTERRACIAL BOUNDARY, LISTED IN GEOGRAPHICAL ORDER FROM NORTH TO SOUTH: DATA OF 1955 AND 1956

17-chromosome Race				15-chromosome Race			
Locality	Sample Size	Blundell	Tidbinbilla	Locality	Sample Size	Blundell	Tidbinbilla
Frogmore	3	—	—				
Boorowa "B"	44	0·455	—	Rugby, 2 miles SE. of	8	0·625	—
Boorowa "A"	60	—	—				
Rye Park, 1·5 miles NNE. of	6	0·550	—				
Rye Park "B"	3						
Rye Park "A"	21						
				Rye Park, 6 miles S. of	8	0·750	—
Tangmangaroo, 5 miles N. of	11	0·273	—	Pudman Creek, 1 mile S. of	7	0·429	—
Yass, 9·5 miles N. of	4	0·361	—	Yass, 10·9 miles N. of	14	0·179	—
Yass, 9·2 miles N. of	17						
Yass, 9 miles N. of	65			Dalton "B"	9	0·214	0·036
Thieves' Creek "B"	11			Dalton "A"	5		
				Yass, 10 miles NNE. of	18	0·417	0·194
				Thieves' Creek "A"	13	0·461	—
Yass, 6 miles NNW. of	6	0·833	0·167				
Bowning "A"	54	0·833	0·111	Jerrawa	5	0·400	—
Bowning, 2 miles ESE. of	6	0·917	0·167	Yass cemetery ..	24	0·479	0·104
Black Range "B"	56	0·688	0·071				

part of the area under consideration (below the dotted line in Fig. 1) and in this area it is found on both sides of the interracial boundary; (2) unusually high frequencies of the Blundell rearrangement occur in the Bowning–Black Range area; (3) in the Thieves' Creek area the frequency of Blundell seems to be similar on either side of the interracial boundary. In general, it can be said that the interracial boundary

does not seem to correspond with any sharp change in the frequencies of the chromosomal rearrangements; there may have been a fairly steep cline in the frequency of Blundell across the interracial boundary between Yass and Bowning, but equally steep clines must have occurred within the territories of the "pure" races.

III. Discussion

It seems virtually certain from the data presented above that no real gap could have existed 100 years ago between the distribution areas of the two races. The first question is therefore whether they met in a sharp line or whether there was a zone of overlap, however narrow.

Although *M. scurra* is an insect of very low mobility, we may take it as axiomatic that any mobility at all would have tended to produce a zone of overlap under the conditions of a semi-continuous population existing prior to the introduction of sheep (by "semi-continuous" we do not imply any discontinuity between the two races but simply that, since the species does not exist in pure dry or wet sclerophyll forest but only in grassland, savannah woodland, and in the ecotones between these and sclerophyll forest, its distribution must always have surrounded islands of irregular shape where it was absent).

The evidence suggests, however, that the zone of overlap was quite narrow, perhaps only a few hundred yards and at most 3–4 miles wide. Such a situation would be explicable if the interracial boundary line corresponded to some sharp ecological discontinuity and if "fused *AB*" chromosomes were adapted to one set of ecological conditions and "broken *AB*" chromosomes to another. However, there is no evidence for any such sharp ecological discontinuity. In a general sense the 17-chromosome race is found in a region of lower rainfall than is inhabited by the 15-chromosome race. Meteorological records are inadequate to determine whether the interracial boundary does, in fact, follow an isohyet. Certainly, however, there are no easily discernible ecological differences of a significant nature between pairs of localities such as Pudman Creek and Rye Park, or the two Thieves' Creek sites. It seems impossible, therefore, that selection against homozygotes (i.e. against fused *AB* homozygotes to the west of the interracial boundary and against broken *AB* homozygotes to the east of it) could have operated to keep the zone of overlap as narrow as it is.

The only remaining explanation for the narrowness of the zone of overlap—and the only one which seems to fit the evidence—is selection against the heterozygotes ("negative heterosis"). It will be clear that such selection would be far more effective in keeping the zone of overlap narrow than selection which operated only at the homozygous level, unless the ecological transition was extraordinarily sharp.

Negative heterosis might have operated to produce monomorphism for the *AB* chromosome in colonies such as Thieves' Creek "B", 9 miles N. of Yass, and 10.9 miles N. of Yass *since* geographic continuity between the races was interrupted by grazing. But this seems unlikely, unless the frequency of the rarer karyotype was already very low. We may consider the possibility that there was a stable polymorphism in respect of fused *v.* broken *AB* chromosomes at these localities 100 years ago (i.e. that the "zone of overlap" was broader than the 1000 yd postulated earlier). In

this case the rate of immigration of the rarer karyotype into each locality would be equal to its rate of elimination by natural selection. The rate of immigration, with an insect of such feeble mobility, would be so low that the colonies would have become monomorphic for the *AB* if the fitness of the heterozygotes was considerably below that of the homozygotes. But if the negative heterosis of the heterozygotes only involved a slight reduction in fitness (say by 5 per cent., which is the figure assumed by Haldane (1942) when considering this question in relation to *Rhrh* heterozygotes in man), then there is no reason to suppose that the colonies mentioned above would have become monomorphic for the *AB* in the 50–100 generations which have elapsed since the continuity of the races was interrupted by destruction of the habitat. Therefore, we consider it likely that the populations at these localities were monomorphic in respect of the *AB* chromosome 100 years ago, and that even at a time when the races were continuous in the Thieves' Creek area, the zone in which fused and broken *AB* chromosomes coexisted was not more than 1000 yd wide.

We are accordingly faced with several subsidiary problems: (1) How does selection against fused/broken *AB* heterozygotes actually operate: (2) how could the 17-chromosome race have established itself in the first place: and (3) how much exchange of genetic material has, in fact, taken place across the interracial boundary?

The first question can be answered, in part, as a result of studies on the cytology of interracial hybrids (White 1957*a*). These studies have shown that such hybrids must have their fertility slightly reduced, particularly if their *CD* chromosome pair is structurally heterozygous (i.e. Standard/Blundell). The single natural chromosome-number heterozygote found at Thieves' Creek "A" did not provide any evidence on this point, since it was rather senile and only showed six first metaphases in all of which meiosis was entirely normal. Studies on experimental interracial hybrids suggests that their fertility must be quite high—perhaps 98 or 99 per cent. of normal in many individuals. In a species with such feeble powers of dispersal as *M. scurra* it is conceivable that a reduction of fertility by even as little as 2 per cent. might keep the zone of overlap very narrow. But it certainly would be easier to understand the narrowness of the overlap zone in *M. scurra* if chromosome-number heterozygosity lowered viability as well as fecundity.

It now seems certain that the 17-chromosome race arose from the 15-chromosome one, rather than vice versa. This event presumably took place somewhere on the western fringe of the species distribution and probably in a quite strongly isolated colony (otherwise the dissociation would hardly have been able to establish itself). The 17-chromosome race, carrying with it the same adaptive polymorphisms (color pattern variability and chromosomal rearrangements), was then able to invade an area somewhat more arid than that of the tableland. As it expanded into this area, previously unoccupied by the species, its advance was probably more in breadth than in depth (as it advanced in depth it would encounter progressively greater aridity). We were unable to find the species at Stockinbingal, Temora, Marsden, and Grenfell, and it probably does not extend far to the westward of a line between Young and Cootamundra.

The establishment of the interracial boundary must have resulted from the "lateral expansion" of the 17-chromosome race from a single point of origin on, or very

close to the periphery of the species distribution. In this way a colony containing a majority of dissociation homozygotes could have arisen rapidly and could have persisted without being swamped by immigration of fused *AB* homozygotes from all sides. There is no evidence that the interracial boundary has advanced or receded since its original establishment, but the possibility of slight advances or recessions cannot be excluded (a few hundred yards in a century would be the maximum one could postulate).

One effect of the dissociation of the *AB* chromosome in *M. scurra* was to increase the amount of genetic recombination, which is consequently higher in the 17-chromosome race than in the 15-chromosome one (see White 1957*a*, Table 1). The chiasma frequency of the *A* and *B* bivalents in the western race is about 50 per cent. greater than that of the *A* and *B* limbs of the fused *AB*. Moreover, while the proximal regions of the *AB* chromosome have a negligible chiasma frequency, the corresponding sections of the separate *A* and *B* chromosomes have quite a high chiasma frequency. Thus the *AB* bivalent almost always shows a single distal chiasma in each arm, while the separate *A* and *B* bivalents each quite frequently have two chiasmata. Whether the very first dissociation homozygotes showed this increase in chiasma frequency is quite unknown. But even if it developed later, as a result of selection, it is very probable that a relatively rapid release of variability, due to the breaking up of close linkage in the middle section of the large *AB* chromosome, helped to adapt the 17-chromosome race to new environmental conditions.

The dissociations of the *AB* found in the heterozygous condition in six individuals of the eastern race collected in 1955 (two at Komungla "B", two at Paddy's River "B", one at Royalla "A", one at Michelago) must now be interpreted as independent occurrences, not identical with the dissociation which gave rise to the western 17-chromosome race. It seems impossible to tell whether the dissociations in these four populations had a single common origin or a multiple one. In addition, the peculiar translocation found in a single member of the Hall population (White 1956, Fig. 4*b*) represents yet another chromosomal rearrangement involving breakage of the *AB* close to the centromere. It is possible that some of these dissociations might have been evolutionary successes if only they had occurred on the periphery of the species distribution instead of in the middle of it. In view of the rarity of these dissociations in the populations of the 15-chromosome race it is most unlikely that they show positive heterosis. But, equally, it is hardly likely that they would have persisted at all if they showed strong negative heterosis.

The extent to which exchange of genetic material has taken place across the interracial boundary cannot be answered from the available evidence. If it could be shown that the populations opposite one another on either side of the interracial boundary were invariably similar morphologically or in cytogenetic constitution, that would constitute presumptive evidence for gene exchange. But the evidence is adequate, although on general grounds it seems clear that a certain amount of genetic exchange must have occurred.

A number of instances are now known where a species may be subdivided into two races differing in chromosome number in such a manner that a pair of metacentric chromosomes in one race is represented by two pairs of acrocentrics in another. In

most of these a zone of overlap seems to occur within which chromosome-number heterozygotes are found. These cases can probably be arranged in a series, according to the degree of heterosis (positive or negative) exhibited by the heterozygotes. At one end of the scale may be placed the mantid *Ameles heldreichi* (Wahrman 1954) where the "zone of overlap" may well be coextensive with the entire species distribution and in any case includes numerous localities from Palestine to Turkey. Here the heterozygotes probably show relatively strong heterosis, so that cytologically monomorphic colonies do not occur, or are rare, and the polymorphism is very widespread. The grasshopper *Trimerotropis sparsa* (White 1951) is normally a 23-chromosome species, but in north-eastern Colorado there is a 21-chromosome race, which has presumably arisen through a centric fusion (i.e. the evolutionary change in chromosome number has been a decrease rather than an increase). The zone of overlap in this case has not been surveyed in detail, but includes Rifle, Colorado, and Duchesne, Utah, so that it is probably fairly broad. Similar zones of overlap probably occur in the grasshoppers *Circotettix undulatus* (Evans 1954) and *Hesperotettix viridis* (McClung 1917) but in these instances it is not possible to determine the extent of the overlap zones for lack of evidence. The zone of hybridization between *Drosophila americana americana* and *D. a. texana*, which also differ in respect of a fusion, is probably several hundred miles wide (Patterson and Stone 1952). At the other extreme of the series from *A. heldreichi* is the *M. scurra* case, where the zone of overlap is (or may be presumed to have been) about as narrow as can be imagined.

Just where in our hypothetical series the case of the mollusc *Purpura lapillus* (Staiger 1954) fits—or whether it fits into this series at all—seems uncertain. In this species there are no less than five "fusions" so that the cytologically monomorphic colonies have either $n = 13$ or $n = 18$, according to whether they are homozygous for all five of these or for none of them. The polymorphic colonies naturally have intermediate chromosome numbers and the individuals in them show from 0 to 5 trivalents at meiosis. According to Staiger, populations homozygous for all five fusions occur in intertidal habitats with a rich supply of food and where wave action is strong; while populations with no fusions occupy localities sheltered from wave action, but where food is scarcer. The polymorphic populations are said to live in the ecologically intermediate habitats. Here it does sound as if the ecological transition might be extremely sharp, so that the interpretation which we have rejected in *M. scurra* (namely strong selection against each homozygous type in the territory of the other) may apply. Such an interpretation is, of course, by no means incompatible with heterosis of the heterozygotes in the intermediate zone.

It would be interesting to be able to relate these various cases to stages in evolutionary divergence or speciation. Sharp positive heterosis of chromosome-number heterozygotes (such as presumably exists in *A. heldreichi*) should act as a unifying force—if the species splits at all it will at any rate not split into two forms differing in chromosome number. Negative heterosis of chromosome-number heterozygotes, which we have postulated in *M. scurra*, should tend to disrupt the species into two, by favoring the accumulation of genetic-isolating mechanisms diminishing interbreeding between the two races. However, there is as yet no evidence that any such isolating mechanisms between the two races of *M. scurra* have been developed.

IV. Acknowledgment

We are particularly indebted to Dr. K. H. L. Key, Division of Entomology, C.S.I.R.O., who realized from the beginning of the work on *M. scurra* that a detailed survey of the geographical junction between the two races would be of critical importance. Dr. Key also visited the Thieves' Creek area with us and helped us to interpret it ecologically.

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CYTOGENETICS OF THE GRASSHOPPER *MORABA SCURRA*

IV. HETEROZYGOSITY FOR "ELASTIC CONSTRICTIONS"

By M. J. D. White*

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Summary

In some populations of *Moraba scurra* Rehn, individuals occur which are heterozygous for chromosomal regions ("elastic constrictions") that become greatly stretched on the spindle of the first meiotic division. Such individuals are always rare (maximum frequency about 1 per cent.) and no individuals homozygous for constrictions have been seen. There are at least three different constrictions—one in the *AB* chromosome, one in the *EF* element, and a third in one of the four short chromosomes. The persistence of these structures, which may be short heterochromatic segments of a special type, argues that they are adaptive; but their rarity suggests that homozygotes have a very low viability or fecundity. Similar constrictions have been found in two other species of Morabinae.

I. Introduction

Genetic variability is of several different types. Some of these are ill-defined or not properly understood as yet. On the one hand, we have "major" structural rearrangements such as inversions and translocations. On the other hand, there are the "point mutations", not detectable cytologically, even in the salivary-gland chromosomes of the Diptera, although they may also be "structural rearrangements"—but of a different order of magnitude. Somewhere between these two main categories lie a variety of "minute" changes. Certain of these are undoubtedly very short inversions, deletions, duplications, or other types of structural change. Others may be point mutations that do express themselves in a cytologically visible manner, e.g. by the "heterochromatinization" of a band in the polytene chromosomes. Minor changes in heterochromatic regions are perhaps especially frequent.

In the genus *Drosophila*, many, but by no means all, species show cytological polymorphism for paracentric inversions. Although genic polymorphism is undoubtedly quite well developed in this genus, there are only a few species such as *D. montium* and *D. polymorpha* in which it leads to conspicuous differences of color pattern or other features of the external phenotype. On the other hand, natural polymorphism for minute structural changes in the salivary-gland chromosomes is apparently rare in *Drosophila*, although in *D. ananassae* minute deficiencies or duplications may be present or absent at the distal ends of some of the chromosomes (Kikkawa 1937). In the fungus midges of the genus *Sciara* minute rearrangements involving one to three bands in the salivary chromosomes are quite common in the natural populations of some species (Metz 1941) and large paracentric inversions also occur in certain members of this genus, just as in *Drosophila* (Carson 1944; McCarthy 1945).

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In most species of grasshoppers (a term which we use here in a broad sense to include the families Acrididae, Eumastacidae, and Tetrigidae) phenotypic polymorphism is usually much more highly developed than in *Drosophila* spp., or, at any rate, is expressed in ways which are more conspicuous to the human eye. Attempts to classify the color patterns in particular species have been made by Rubtzov (1935), Key (1954), and Richards and Waloff (1954)—but it is only in the grouse locusts (Tetrigidae) that a detailed genetic analysis of the color patterns has been carried out (Nabours and Stebbins 1950 and earlier papers).

In the Australian species *Moraba scurra* Rehn (Orthoptera: Eumastacidae) there is one very conspicuous polymorphism which is limited in its expression to the female, some individuals of that sex being green, either with or without a dorsal stripe of some other color (no green males occur). But, in addition, there are many other colors and color patterns which express themselves in both sexes. The inheritance of these has not been worked out, but they are presumably simple mendelian differences, depending on point mutations.

The major cytological polymorphisms of *M. scurra* consist of three alternative gene sequences for the *CD* chromosome and two for the *EF* chromosome (White 1956, 1957*a*, 1957*b*; White and Chinnick 1957). These are apparently related to one another, in the case of each chromosomal element, as pericentric inversions; they give rise to heterotic effects on viability, when heterozygous. Whether they also enhance fertility is not known.

In addition to these two main types of genetic polymorphism (both of which must surely play a major role in the population genetics of the species) a third, but quite rare, kind of polymorphism exists. Certain individuals are heterozygous for chromosomal regions which become extraordinarily elongated and stretched on the spindle of the first meiotic division, presumably becoming unspiralized under tension. Since the presence of these "elastic regions" is not associated with any major inversion or other rearrangement, they must be classified as a special type of minute structural change (although they may be several microns in length when fully stretched).

A phenomenon which is probably similar in nature has long been known in certain species of true grasshoppers (family Acrididae); but since the degree of elongation is much less, the chromosomes merely show a slight narrowing or constriction, or, in some instances, a short unstained gap. Thus Helwig (1955), speaking of *Circotettix verruculatus*, says: "Occasionally tetrad 4 will have one or both diads 'knobbed' owing to a constriction near the proximal end . . . The frequency of 'Knobbed' chromosome 4 is very small." These constrictions, which have been seen by the author in several species of Acrididae, are relatively inconspicuous and even difficult to score reliably; on the other hand, all those that have been seen in the Morabinae become so greatly stretched on the spindle at the first meiotic division that they are very obvious.

II. Observations

Our records on the occurrence of elastic constrictions in the chromosomes of *M. scurra* are given in Table 1. It is possible that a few additional instances went unnoticed, since the primary object of the cytological study was to obtain data on

the chromosomal rearrangements; however, the table provides substantially accurate information on the frequency of the constrictions in the natural populations. It is uncertain whether the constriction that was found once in an *EF* chromosome with the Tidbinbilla rearrangement is the same one that was encountered in an *EF* with the Standard sequence, so that we cannot be certain whether there are three or four different constrictions in the species (the constrictions seen in the *AB* are all believed to be at the same locus, as are those in the small chromosome). All our records of these constrictions relate to the 15-chromosome race, none having been encountered in 1851 individuals of the 17-chromosome race that have been examined cytologically.

TABLE 1

INDIVIDUALS HETEROZYGOUS FOR CONSTRICTIONS: DATA OF 1955–1957

Locality	Sample Size	Chromosome			
		AB	*EF* (Standard)	*EF* (Tidbinbilla)	One of Four Small Pairs
Paddy's River "B"	200	—	—	—	1
Komungla "B"	178	1	—	—	—
Schofield	84	1	—	—	1
Hall	1377	2	—	—	—
Royalla "A"	725	—	—	1	—
Royalla "B"	600	1	—	—	—
Michelago	550	—	1	—	—
Williamsdale	300	3	—	—	—
Yass, 10 miles NNE. of	18	—	—	—	1
Pudman Creek	7	1	—	—	—
Lyndhurst	14	—	—	—	1
Other localities	3981	—	—	—	—
Totals	8034	9	1	1	4

The elastic constrictions do not seem to be evident in spermatogonial metaphases, or are, at any rate, not easily visible at that stage. At diakinesis, however, the constrictions are in most instances quite conspicuous, there being a short unstained gap in the chromosome to mark their position. As soon as a bivalent heterozygous for a constriction is stretched on the spindle at first metaphase, this gap becomes enormously stretched if, as is almost always the case in *M. scurra*, there is a chiasma distal to it. The longest constriction, when fully extended on the spindle, is that found in the small acrocentric chromosome pair (Fig. 1(*c*)); its length may be several times that of the rest of the chromosome. In the case of the *AB* bivalent, the presence of a chiasma between the arms not heterozygous for a constriction usually prevents the constriction in the other arm from being maximally stretched.

However elongated these elastic regions may become, there is no evidence from a careful study of anaphases and telophases that they ever undergo breakage at the first meiotic division. Thus eventually the terminalized chiasmata become resolved

and the portions distal to the stretched constriction rejoin the proximal part of the chromosome in late anaphase or telophase, as the constriction contracts again. The course of events, as far as the *AB* bivalent is concerned, is well shown in the photographs of early and late anaphases (Plate 1, Figs. 3 and 4).

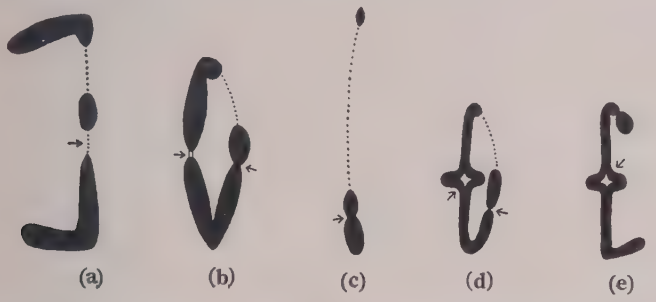

Fig. 1.—Bivalents heterozygous for elastic constrictions (slightly schematized). (*a*) the *AB* bivalent of *Keyacris* sp.; (*b*) that of *M. scurra*; (*c*) one of the small bivalents of *M. scurra*; (*d*) and (*e*) the *CD* bivalent of *Callitala brevicornis*. In (*d*) there are chiasmata in both arms, while in (*e*) there is no chiasma in the short arm. Positions of chiasmata indicated by small arrows.

Elastic constrictions of the same general type have been seen in two other members of the subfamily Morabinae. A single individual of an undescribed species of *Keyacris*, from a locality 3 miles W. of Cobbadah, N.S.W. (one of 10 examined cytologically), was heterozygous for a constriction in the *AB* chromosome (Fig. 2(*a*)), quite similar to that known in *M. scurra*. And one of two nymphs provisionally

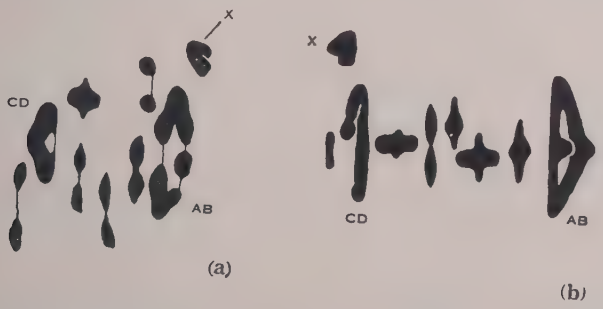

Fig. 2.—First metaphases, in side view, of individuals heterozygous for elastic constrictions. (*a*) *Keyacris* sp. (3 miles W. of Cobbadah, N.S.W.) with a constriction in an *AB* chromosome; (*b*) *Callitala brevicornis* with a constriction in a *CD* chromosome (8 miles SE. of Larrimah, N.T.). Figure 2 (*b*) may be compared with Plate 1, Figure 5, which is a photograph of the same cell.

assigned to *Callitala brevicornis* (Walker), collected 8 miles S. of Larrimah, N.T., was heterozygous for a constriction in the *CD* chromosome (Figs. 1(*d*), 1(*e*), 2(*b*); Plate 1, Fig. 5). Four additional individuals of the same species from the Katherine–Darwin area were cytologically normal. These *Keyacris* and *Callitala* constrictions are quite comparable in their degree of extension at first metaphase with those of *M. scurra*; since only small numbers of these species have been examined cyto-

logically, there seems to be a distinct possibility that their constrictions may be present in higher frequency than in *M. scurra*.

The constriction in the *CD* chromosome of *C. brevicornis* is in the short arm of that chromosome and appears quite differently (Figs. 1(*d*) and 1(*e*)) according to whether or not there is a chiasma in that arm; if there is no chiasma the constriction is not stretched on the metaphase spindle and is relatively inconspicuous.

III. Discussion

It must remain uncertain for the present whether the elastic constrictions of the Morabinae are single genetic loci or longer chromosomal segments, possibly analogous to the heterochromatic segments of some plants such as *Trillium* and *Paris*, which are revealed by cold treatment, and for which individual plants are frequently heterozygous (Haga and Kurabayashi 1954; Haga 1956; Rutishauser 1956). Also we do not know whether the morabine constrictions are represented by a homologous, but non-elastic, section in the normal chromosome or not. If there is no corresponding segment in the normal chromosome, the elastic regions would be extra segments inserted into the chromosome and we should have to consider where they might have originated. It is conceivable, but perhaps somewhat unlikely, that the three elastic segments known in *M. scurra* are actually homologous, in the sense of having had a common origin. But our data on the constriction of the *AB* chromosome (which seems to occupy a constant position) do not suggest that this is a "movable" structure like the *Dissociation* and *Activator* loci of maize (McClintock 1953).

The degree of elongation of these elastic constrictions at first metaphase is so great that we feel sure they must be unspiralized at this stage. But the fact that they are already visible at diakinesis shows that it is not tension alone which leads to their unspiralization. And whatever the precise molecular structure of these regions, it is certainly one with a considerable tensile strength.

The presence of the elastic constrictions (in very low frequency, it is true) in most of the larger colonies of the 15-chromosome race which were examined argues either (1) that they have some positive adaptive property; (2) that they are arising *de novo* at a rate which would compensate for their loss, assuming them to be non-adaptive; or (3) that they are favored by some kind of "meiotic drive" (Sandler and Novitski 1957).

There is no direct evidence for any of these alternatives. The observations on spermatogenesis seem to exclude the possibility of meiotic drive as far as the male is concerned; but it could still exist in the female meiosis. Fairly frequent *de novo* origin seems unlikely on general grounds, and the apparent absence of elastic constrictions from the 17-chromosome race of *M. scurra* is more easily explained by assuming that they have simply never gotten into this race than by supposing the race to lack a mutational ability which the 15-chromosome form possesses. We are hence inclined to favor the hypothesis that individuals heterozygous for elastic constrictions do enjoy some selective advantage, although the low frequency of chromosomes carrying these structures, in the natural populations, suggests that the homozygotes may have a very low selective value, approaching or reaching zero (absolute lethality or sterility).

A selective advantage could be due to qualities inherent in the elastic segment itself, or to genetic loci closely linked with it. It would hardly be surprising if such a segment, when heterozygous, acted as an absolute crossover suppressor for a short region on either side of itself. If so, any loci within that region would be completely linked to the elastic constriction.

Although we have never seen a homozygote for an elastic constriction, this does not prove that they are inviable, in view of the very low frequency of heterozygotes. Thus at Williamsdale, where the frequency of individuals heterozygous for the elastic constriction in the *AB* chromosome is 1 per cent., we should expect one homozygote in 40,000 individuals if the whole colony is a single panmictic unit, or a somewhat higher frequency (say from one in 5000 to one in 10,000) if there is some degree of local inbreeding.

The first anaphase disjunction of bivalents heterozygous for elastic constrictions seems to be invariably regular, and the segment distal to the constriction rejoins the main part of the chromosome as the constriction contracts at telophase (Plate 1, Figs. 3 and 4). However, it is by no means certain that the anaphase disjunction of a bivalent that was homozygous for such a constriction would be equally regular. In fact, it is quite possible that in such a bivalent the two segments distal to the constrictions would become left in the middle of the elongating spindle at first telophase and that when cytokinesis took place they might often both get included in the same cell. If so, homozygotes for elastic constrictions would be highly sterile. While such an interpretation must remain hypothetical until the meiosis of a constriction-homozygote has actually been observed, it would certainly help to explain why all the elastic constrictions—at any rate in *M. scurra*—are present in such low frequency in the natural populations.

IV. Acknowledgment

I am greatly indebted to Dr. K. H. L. Key, Division of Entomology, C.S.I.R.O., for the material of *Keyacris* and *Callitala* collected by him.

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Explanation of Plate 1

Figs. 1 and 2.—First metaphases, in side view, of an individual of *M. scurra* from the Schofield population, heterozygous for an elastic constriction in the *AB* chromosome. In Figure 1 the *AB* bivalent is on the right-hand side of the cell, in Figure 2 it is on the left-hand side of the cell. In both cases it is the upper member of the *AB* pair which has the constriction. Several developing sperms (cigar-shaped, sickle-shaped, or zig-zag objects) are visible in these photographs. From an aceto-orcein squash preparation. This individual had the karyotype *Bl*/*Bl*, *St*/*St*.

Figs. 3 and 4.—First anaphases, in side view, of an individual of *M. scurra* from the Hall, A.C.T., colony, heterozygous for an elastic constriction in the *AB* chromosome. In both cases the *AB* chromosomes are on the right-hand side, in Figure 3 the upper *AB* chromosome still shows the portion distal to the constriction separate from the main part of the chromosome, but at a slightly later stage (Fig. 4) the elastic constriction has contracted so that it is no longer easily visible. Aceto-orcein squashes.

Fig. 5.—First metaphase in an individual of *Callitala brevicornis*, heterozygous for an elastic constriction in the *CD* chromosome. Compare text-figure 2(*b*).

CYTOGENETICS OF THE GRASSHOPPER MORABA SCURRA. IV